

of afucosylation (i.e., the lack of core fucose on the N-linked glycan) can be highly desirable for a mAb product with ADCC as a mechanism of action. Strategies to modulate mAb glycosylation (e.g., galactosylation and/or fucosylation) in cell culture processes generally belong to one of four categories: (1) genetic engineering of recombinant cell lines (Louie et al., (2016), *Biotechnol Bioeng*, 114 (3), 632-644; Yamane-Ohnuki et al., (2004), *Biotechnol Bioeng*, 87(5), 614-622); (2) addition of enzyme inhibitors (Allen et al., (2016), *ACS Chem Biol*, 11(10), 2734-2743; Okeley et al., (2013), *PNAS*, 110 (14), 5404-5409); (3) modifying the levels of co-factors and substrates for glycosylation, including supplementation with alternative sugars (Hossler et al., (2014), *Biotechnol Prog*, 30 (6), 1419-1431; Hossler et al., (2017), *mAbs*, 9 (4), 715-734); and (4) fine-tuning of culture process parameters (Konno et al., (2012), *Cytotechnology*, 64, 249-265). Although many cell culture process parameters are known to impact galactosylation (Hossler et al., (2009), *Glycobiology*, 19 (9), 936-949), fewer have been identified to control fucosylation.

15

SUMMARY

The subject matter disclosed herein relates to modulating the glycosylation pattern (e.g., galactosylation and/or fucosylation pattern(s)) of a recombinant glycoprotein of interest. For example, but not by way of limitation, the examples described herein relate to modulating glycosylation to achieve or preserve a desired glycoprotein glycosylation pattern (e.g., galactosylation and/or fucosylation pattern(s)). Methods by which glycosylation can be modulated in accordance with the instant disclosure include, but are not limited to: (1) control of cell culture media and/or cell culture manganese (Mn) concentration, e.g., with respect to Mn concentration analysis of raw materials, Mn supplementation to cell culture media and/or during cell culture, and/or minimizing Mn loss from cell culture by establishing a reduced pH target or set point for media pH adjustment prior to High Temperature Short Time (HTST) heat treatment of the media; (2) controlling process parameters during cell culture, e.g., pCO₂, media hold duration, culture duration, cultivation temperature and osmolality/Na⁺; and (3) control of cell culture media and/or cell culture galactose and/or fucose concentration. The subject matter of the present disclosure is also directed to cell culture and glycoprotein compositions prepared when such process parameters are controlled as described herein.

In one aspect, the present disclosure refers to a method for modulating the

fucosylation pattern of an antibody or antibody fragment in a cell culture, comprising:
modulating in a cell culture medium:

- a. a manganese (Mn) concentration from 1 nM to 20000 nM in a high partial pressure CO₂ (pCO₂) condition, wherein said high pCO₂ condition is from 20 to 250
5 millimeters of mercury (mmHg); and
 - b. a Mn concentration from 1 nM to 30000 nM in a low pCO₂ condition, wherein said low pCO₂ condition is from 10 to 100 mmHg; and
- wherein said antibody fragment is a scFv (single-chain variable fragment), BsDb (bispecific diabody), scBsDb (single-chain bispecific diabody), scBsTaFv (single-
10 chain bispecific tandem variable domain), DNL-(Fab)₃ (dock-and-lock trivalent Fab), sdAb (single-domain antibody) or a BssdAb (bispecific single-domain antibody).

In another aspect, the present disclosure refers to a method to prepare a cell culture medium, a feed medium, a hydrolysate, or an additive comprising modulating:

- a. the Mn concentration in a high partial pressure CO₂ (pCO₂) culture from 1
15 nM to 20000 nM, wherein said high pCO₂ culture is from 20 to 250 mmHg; and
- b. the Mn concentration in a low pCO₂ culture from 1 nM to 30000 nM, wherein said low pCO₂ culture is from 10 to 100 mmHg;

wherein the cell culture media, feed media, hydrolysate, or additive modulates
20 the fucosylation pattern of an antibody or antibody fragment of interest.

In another aspect, the present disclosure refers to a cell culture composition comprising,

- a. a host cell engineered to express an antibody or antibody fragment of interest; and
- 25 b. a cell culture and/or cell culture media modulated to target one or more predetermined parameter selected from:
 - i. the Mn concentration in a high partial pressure CO₂ (pCO₂) culture from 1 nM to 20000 nM, wherein said high pCO₂ condition is from 20 to 250 mmHg; and

ii. the Mn concentration in a low pCO₂ culture from 1 nM to 30000 nM, wherein said low pCO₂ condition is from 10 to 100 mmHg; and

wherein said antibody fragment is a scFv (single-chain variable fragment), BsDb (bispecific diabody), scBsDb (single-chain bispecific diabody), scBsTaFv (single-chain bispecific tandem variable domain), DNL-(Fab)₃ (dock-and-lock trivalent Fab),
5 sdAb (single-domain antibody) or a BssdAb (bispecific single-domain antibody).

In another aspect, the present disclosure refers to a method for producing a glycoprotein of interest in a cell culture, comprising:

- a. subjecting a cell culture medium suitable for cultivating a eukaryotic cell
10 to the method as described herein,
- b. inoculating the modulated cell culture medium with the eukaryotic cell that expresses the recombinant protein;
- c. cultivating the eukaryotic cell so that the recombinant protein is expressed.

In certain examples, the present disclosure is directed to a method for

15

What is claimed is:

1. A method for modulating the fucosylation pattern of an antibody or antibody fragment in a cell culture, comprising:

modulating in a cell culture medium:

- a. a manganese (Mn) concentration from 1 nM to 20000 nM in a high partial pressure CO₂ (pCO₂) condition, wherein said high pCO₂ condition is from 20 to 250 millimeters of mercury (mmHg); and
- b. a Mn concentration from 1 nM to 30000 nM in a low pCO₂ condition, wherein said low pCO₂ condition is from 10 to 100 mmHg; and

wherein said antibody fragment is a scFv (single-chain variable fragment), BsDb (bispecific diabody), scBsDb (single-chain bispecific diabody), scBsTaFv (single-chain bispecific tandem variable domain), DNL-(Fab)₃ (dock-and-lock trivalent Fab), sdAb (single-domain antibody) or a BssdAb (bispecific single-domain antibody).

2. The method of claim 1, wherein the duration of pCO₂ modulation covers at least the first half of the cell culture duration.

3. The method of claim 1, wherein the antibody is a chimeric, a humanized or a human antibody, optionally wherein the antibody is an anti-CD20 antibody, optionally wherein the anti-CD20 antibody is ocrelizumab.

4. The method of claim 1, wherein the antibody or antibody fragment exhibits:

- i) a % afucosylated glycoprotein between 0% to 20%; 1% to 15%; 1% to 10%; or 1% to 8%; or, a normalized % afucosylated glycoprotein between 0% to 20%; 1% to 15%; 1% to 10%; or 1% to 8%; and/or,
- ii) a % agalactosylated glycoprotein between 40% to 90%; 50% to 90%; 55% to 85%; or 60% to 80%.

5. The method of claim 1, wherein the fucosylation is modulated to achieve:

- a. increased afucosylation, while decreasing agalactosylation; or,
- b. decreased afucosylation, while increasing agalactosylation; or,
- c. increased or decreased afucosylation without impacting agalactosylation.

6. The method of claim 1, wherein modulation of the Mn concentration comprises determining the Mn content in cell culture raw materials and selecting raw material lots to modulate the Mn concentration, optionally wherein the modulation of the Mn concentration comprises employing a cell culture media pH of 6.1 to 7.3; or 6.3 to 7.3 prior to High Temperature Short Time heat

treatment, optionally wherein modulation of the Mn concentration comprises (i) controlling materials that come into contact with cell culture media or cell culture; or (ii) accounting for the concentration of leached Mn in cell culture media or during cell culture; or a combination of (i) and (ii) to modulate the Mn concentration, optionally wherein the leached Mn is produced by contact of the cell culture and/or cell culture media with: (i) a filter; (ii) a media preparation, hold, or culture vessel; or (iii) combinations of (i) and (ii).

7. The method of claim 1, wherein the cell culture medium is a basal medium, a reconstituted medium, a feed medium, a hydrolysate, a supplement, serum or an additive, optionally wherein the cell culture medium is supplemented during or prior to the production stage of the cell culture, optionally

wherein the cell culture medium comprises Mn, and wherein the supplementation is based on a pre-defined schedule or criteria, optionally wherein the Mn is supplemented as a bolus, as an intermittent supplement, as a continuous supplement, as a semi-continuous supplement, as a feedback loop-based supplement, or as a combination of one or more of thereof.

8. The method of claim 1, wherein the cell culture medium consists essentially of Mn.

9. The method of claim 1, wherein modulation of the fucosylation pattern of the glycoprotein of interest comprises modulating the pCO₂, optionally wherein the pCO₂ modulation comprises establishing a low pCO₂ culture, optionally wherein the pCO₂ modulation occurs at day 0 of the culture, wherein said low pCO₂ is from 10 to 100 mmHg.

10. The method of claim 1, wherein the cell culture comprises eukaryotic cells, optionally wherein the eukaryotic cells are CHO cells.

11. The method of claim 1, wherein the cell culture is in a bioreactor, wherein the bioreactor comprises a single use technology (SUT) bag or bioreactor; a WAVE bioreactor; a stainless steel bioreactor; a flask; a tube and a chamber, optionally wherein the volume of the cell culture is from 1 mL to 35,000 L.

12. A method to prepare a cell culture medium, a feed medium, a hydrolysate, or an additive comprising modulating:

a. the Mn concentration in a high partial pressure CO₂ (pCO₂) culture from 1 nM to 20000 nM, wherein said high pCO₂ culture is from 20 to 250 mmHg; and

b. the Mn concentration in a low pCO₂ culture from 1 nM to 30000 nM, wherein said low pCO₂ culture is from 10 to 100 mmHg ,

wherein the cell culture media, feed media, hydrolysate, or additive modulates the fucosylation pattern of an antibody or antibody fragment of interest.

13. A cell culture composition comprising,

a. a host cell engineered to express an antibody or antibody fragment of interest; and

b. a cell culture and/or cell culture media modulated to target one or more predetermined parameter selected from:

- i. the Mn concentration in a high partial pressure CO₂ (pCO₂) culture from 1 nM to 20000 nM, wherein said high pCO₂ condition is from 20 to 250 mmHg; and
- ii. the Mn concentration in a low pCO₂ culture from 1 nM to 30000 nM, wherein said low pCO₂ condition is from 10 to 100 mmHg; and

wherein said antibody fragment is a scFv (single-chain variable fragment), BsDb (bispecific diabody), scBsDb (single-chain bispecific diabody), scBsTaFv (single-chain bispecific tandem variable domain), DNL-(Fab)₃ (dock-and-lock trivalent Fab), sdAb (single-domain antibody) or a BssdAb (bispecific single-domain antibody).

14. A method for producing a glycoprotein of interest in a cell culture, comprising:

- a. subjecting a cell culture medium suitable for cultivating a eukaryotic cell to the method according to claim 1,
- b. inoculating the modulated cell culture medium with the eukaryotic cell that expresses the recombinant protein;
- c. cultivating the eukaryotic cell so that the recombinant protein is expressed.