

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.

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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.

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In one aspect, the present disclosure refers to a method for producing a glycoprotein of interest in

a cell culture, wherein the glycoprotein of interest is ocrelizumab which exhibits a normalized % G0-F (percent afucosylated glycoprotein) of 1% to 8%, the method comprising:

- 5 a. subjecting a cell culture medium suitable for cultivating a eukaryotic cell to a method for modulating the glycosylation pattern of ocrelizumab, the method comprising controlling the cell culture manganese (Mn) concentration between 20 nM and 300 nM;
- b. inoculating the modulated cell culture medium with the eukaryotic cell that
- 10 expresses ocrelizumab;
- c. cultivating the eukaryotic cell so that ocrelizumab is expressed.

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

- a. a host cell engineered to express ocrelizumab; and
- 15 b. a cell culture modulated to control the cell culture manganese (Mn) concentration between 20 nM and 300 nM, wherein ocrelizumab exhibits a normalized % G0-F (percent afucosylated glycoprotein) of 1% to 8%.

In certain examples, the cell culture environment is in a bioreactor with or without cells. In certain examples, the low pCO₂ condition is from about 10 to about 100 mmHg, and the high pCO₂ condition is from about 20 to about 250 mmHg.

- 5 In certain examples, the duration of pCO₂ modulation covers at least the first half of the cell culture duration.

In certain examples, the glycoprotein of interest is a recombinant protein. In certain examples, the recombinant protein is an antibody or antibody fragment, 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) and BssdAb (bispecific single-domain antibody). In certain examples, the antibody is a chimeric, a humanized or a human antibody. In certain examples, the antibody is an anti-CD20 antibody. In certain examples, the anti-CD20 antibody is ocrelizumab. In certain examples, the antibody or antibody fragment exhibits: a % G0-F (percent afucosylated glycoprotein) between about 0% to about 20%; about 1% to about 15%; about 1% to about 10%; or about 1% to about 8%; or, a normalized % G0-F between about 0% to about 20%; about 1% to about 15%; about 1% to about 10%; or about 1% to about 8%; and/or, a % G0 (percent agalactosylated glycoprotein) between about 40% to about 90%; about 50% to about 90%; about 55% to about 85%; or about 60% to about 80%.

What is claimed is:

1. A method for producing a glycoprotein of interest in a cell culture, wherein the glycoprotein of interest is ocrelizumab which exhibits a normalized % G0-F (percent afucosylated glycoprotein) of 1% to 8%, the method comprising:
 - a. subjecting a cell culture medium suitable for cultivating a eukaryotic cell to a method for modulating the glycosylation pattern of ocrelizumab, the method comprising controlling the cell culture manganese (Mn) concentration between 20 nM and 300 nM;
 - b. inoculating the modulated cell culture medium with the eukaryotic cell that expresses ocrelizumab;
 - c. cultivating the eukaryotic cell so that ocrelizumab is expressed.
2. The method of claim 1, wherein the method comprises controlling the Mn concentration in the cell culture medium between 20 nM and 200 nM, or between 30 nM and 110 nM.
3. The method of claim 2, wherein the method further comprises assaying the cell culture medium to determine if the Mn concentration of the cell culture medium falls between 20 nM and 200 nM, or between 30 nM and 110 nM.
4. The method of any one of claims 1 to 3, wherein ocrelizumab exhibits:
 - i) a normalized % G0-F between 1% to 5%; and/or,
 - ii) a % G0 (percent agalactosylated glycoprotein) between 40% to 90%; 50% to 90%; 55% to 85%; or 60% to 80%.
5. The method of any one of claims 1-4, wherein controlling the cell culture 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 control the Mn concentration.
6. The method of claim 5, 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.
8. The method of any one of the preceding claims, wherein the cell culture medium is supplemented with Mn during the production stage of the cell culture or prior to the production stage of the cell culture.

9. The method of claim 8, wherein the supplementation is based on a pre-defined schedule or criteria.
10. The method of claim 8 or claim 9, 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.
11. The method of any one of claims 1 to 10, wherein controlling 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 (HTST) heat treatment.
12. The method of any one of claims 1 to 11, wherein modulation of the glycosylation pattern of ocrelizumab comprises modulating the pCO₂, optionally wherein the duration of pCO₂ modulation covers at least the first half of the cell culture duration.
13. The method of claim 12, wherein the pCO₂ modulation comprises establishing a pCO₂ culture, between 10 mmHg to 100 mmHg, or occurs at day 0 of the culture.
14. The method of any one of claims 1 to 13, wherein modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the duration of the cell culture, wherein the duration of the cell culture is 0 days to 150 days; 0 days to 15 days; 0 days to 12 days; 0 days to 7 days; or 0 days to 5 days.
15. The method of any one of the preceding claims, wherein modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the Na⁺ concentration, wherein the Na⁺ concentration is 0 mM to 300 mM; is 20 mM to 20 mM; 30 mM to 150 mM; or 40 mM to 130 mM.
16. The method of any one of the above claims, wherein modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the osmolality, wherein the osmolality of is 250 mOsm/kg to 550 mOsm/kg; 300 mOsm/kg to 450 mOsm/kg; or 325 mOsm/kg to 425 mOsm/kg.
17. The method of any one of the preceding claims, wherein modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the galactose concentration, wherein the galactose concentration is 0 mM to 60 mM or 0 mM to 50 mM.
18. The method of any one of the preceding claims, wherein modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the fucose concentration, wherein the fucose concentration is 0 mM to 60 mM; 0 mM to 40 mM; 0 mM to 20 mM; or 0 mM to 10 mM.
19. The method of any one of the preceding claims, wherein modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the cell culture temperature, wherein the cell culture temperature is 29°C to 39°C; 30°C to 39°C; 31°C to 38°C; or 34°C to 38°C.
20. The method of any one of the preceding claims, wherein the cell culture temperature is modulated prior the production stage of the cell culture or based on a pre-defined schedule or criteria.

21. The method of any one of the preceding claims, wherein the eukaryotic cell is a mammalian cell.
22. The method of claim 21, wherein the mammalian cell is a CHO cell.
23. The method of any one of claims 1 to 22, 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.
24. The method of any one of claims 1 to 23, wherein the volume of the cell culture is from 1 mL to 35,000 L.
25. The method of claim 1, wherein the method further comprises obtaining a composition comprising ocrelizumab with a normalized % G0-F of 1% to 8%.
26. A cell culture composition comprising,
- a. a host cell engineered to express ocrelizumab; and
 - b. a cell culture modulated to control the cell culture manganese (Mn) concentration between 20 nM and 300 nM;
- wherein, ocrelizumab exhibits a normalized % G0-F (percent afucosylated glycoprotein) of 1% to 8%.