

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-OF-THE-INVENTION

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 ~~embedimentsexamples~~ 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 modulating the

glycosylation pattern of a glycoprotein of interest in a cell culture, comprising:
modulating in a cell culture medium, and/or, in a cell culture
environment:

- 5 a. a Mn concentration from 1 nM to 20000 nM in a high partial pressure
C0₂ (pC0₂) condition; and
b. a Mn concentration from 1 nM to 30000 nM in a low pC0₂ condition.

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

- 10 a. the Mn concentration in a high partial pressure C0₂ (pC0₂) culture from 1
nM to 20000 nM; and
b. the Mn concentration in a low pC0₂ culture from 1 nM to 30000 nM;

wherein the cell culture media, feed media, hydrolysate, or additive modulates
the glycosylation pattern of a glycoprotein of interest.

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

a. a host cell engineered to express a glycoprotein of interest; and
b. a cell culture and/or cell culture media modulated to target one or more
predetermined parameter selected from:

- 20 i. the Mn concentration in a high partial pressure C0₂ (pC0₂) culture from 1
nM to 20000 nM; and
ii. the Mn concentration in a low pC0₂ culture from 1 nM to 30000 nM.

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

- 25 a. subjecting a cell culture medium suitable for cultivating a eukaryotic cell
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.

- 30 In certain ~~embodiments~~examples, the present disclosure is directed to a
method for

modulating the glycosylation pattern of a glycoprotein of interest in a cell culture, comprising: modulating the following parameters, either alone or in any combination, in a cell culture medium, and/or, in a cell culture environment: a Mn concentration from about 1 nM to about 20000 nM in a high partial pressure CO₂ (pCO₂) condition; a Mn concentration from about 1 nM to about 30000 nM in a low pCO₂ condition; a pCO₂ from about 10 mmHg to about 250 mmHg; a pre-inoculation cell culture media hold duration from about 0 hrs to about 120 hrs at a temperature of about 25°C to 39°C; a cell culture duration from about 0 days to about 150 days; a Na⁺ concentration from about 0 mM to about 300 mM; an osmolality from about 250 mOsm/kg to about 550 mOsm/kg; a galactose concentration from about 0 mM to about 60 mM; a fucose concentration from about 0 mM to about 60 mM; and a cultivation temperature from about 29°C to about 39°C.

In certain embodimentsexamples, the cell culture environment is in a bioreactor with or without cells. In certain embodimentsexamples, 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.

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

In certain embodimentsexamples, the glycoprotein of interest is a recombinant protein. In certain embodimentsexamples, 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 embodimentsexamples, the antibody is a chimeric, a humanized or a human antibody. In certain embodimentsexamples, the antibody is an anti-CD20 antibody. In certain embodimentsexamples, the anti-CD20 antibody is ocrelizumab. In certain embodimentsexamples, 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%.

In certain ~~embodiments~~examples, the glycosylation is modulated to achieve: an increased afucosylation (e.g., G0-F (afucosylated G0)), while decreasing agalactosylation (e.g., G0 (fucosylated, agalactosylated G0)); or, a decreased afucosylation (e.g., G0-F), while increasing agalactosylation (e.g., G0); or, an increased or decreased afucosylation (e.g., G0-F) without impacting agalactosylation (e.g., G0); or, an increased or decreased agalactosylation (e.g., G0) without impacting afucosylation (e.g., G0-F).

In certain ~~embodiments~~examples, the step of modulating the glycosylation pattern of the glycoprotein of interest comprises: modulating the Mn concentration from about 1 nM to about 30000 nM under a low pCO₂ condition, or, modulating the Mn concentration from about 1 nM to about 20000 nM under a high pCO₂ condition, and the following parameters, either alone or in any combination, in the cell culture medium, and/or in the cell culture environment: the pre-inoculation cell culture media hold duration from about 0 hrs to about 120 hrs at a temperature of about 25°C to 39°C; the cell culture duration from about 0 days to about 150 days; the Na⁺ concentration from about 0 mM to about 300 mM; the osmolality from about 250 mOsm/kg to about 550 mOsm/kg; the galactose concentration from about 0 mM to about 60 mM; the fucose concentration from about 0 mM to about 60 mM; and the cultivation temperature from about 29°C to about 39°C.

In certain ~~embodiments~~examples, the step of modulating the glycosylation pattern of the glycoprotein of interest comprises: modulating the Mn concentration from about 1 nM to about 30000 nM under a low pCO₂ condition, or, modulating the Mn concentration from about 1 nM to about 20000 nM under a high pCO₂ condition, and the following parameters in the cell culture medium, and/or in the cell culture environment: the pre-inoculation cell culture media hold duration from about 0 hrs to about 120 hrs at a temperature of about 25°C to 39°C; and the cell culture duration from about 0 days to about 150 days.

In certain ~~embodiments~~examples, the step of modulating the glycosylation pattern of the glycoprotein of interest comprises: modulating the Mn concentration from about 1 nM to about 30000 nM under a low pCO₂ condition, or, modulating the Mn concentration from about 1 nM to about 20000 nM under a high pCO₂ condition, and the following parameters in the cell culture medium, and/or in the cell culture environment: the galactose concentration from about 0 mM to about 60 mM; and/or, the fucose concentration from about 0 mM to about 60 mM.

In certain embodimentsexamples, the step of modulating the glycosylation pattern of the glycoprotein of interest comprises: modulating the pre-inoculation cell culture media hold duration and any of the following parameters, either alone or in any combination, in the cell culture medium, and/or in the cell culture environment: a Mn concentration from about 1 nM to about 20000 nM in a high partial pressure CO₂ (pCO₂) condition; a Mn concentration from about 1 nM to about 30000 nM in a low pCO₂ condition; a pCO₂ from about 10 mmHg to about 250 mmHg; a cell culture duration from about 0 days to about 150 days; a Na⁺ concentration from about 0 mM to about 300 mM; an osmolality from about 250 mOsm/kg to about 550 mOsm/kg; a galactose concentration from about 0 mM to about 60 mM; a fucose concentration from about 0 mM to about 60 mM; and a cultivation temperature from about 29°C to about 39°C; wherein the cell culture media hold duration is from about 0 hrs to about 120 hrs at a temperature of about 25°C to 39°C.

In certain embodimentsexamples, the step of modulating the glycosylation pattern of the glycoprotein of interest comprises: modulating the pre-inoculation cell culture media hold duration and the following parameters in the cell culture medium, and/or in the cell culture environment: the Mn concentration from about 1 nM to about 20000 nM in a high partial pressure CO₂ (pCO₂) condition, or, a Mn concentration from about 1 nM to about 30000 nM in a low pCO₂ condition; the pCO₂ from about 10 mmHg to about 250 mmHg; and, the cell culture duration from about 0 days to about 150 days; wherein the cell culture media hold duration is from about 0 hrs to about 120 hrs at a temperature of about 25°C to 39°C.

In certain embodimentsexamples, the step of modulating the glycosylation pattern of the glycoprotein of interest comprises: modulating the cell culture duration and any of the following parameters, either alone or in any combination, in the cell culture medium, and/or in the cell culture environment: a Na⁺ concentration from about 0 mM to about 300 mM; an osmolality from about 250 mOsm/kg to about 550 mOsm/kg; a galactose concentration from about 0 mM to about 60 mM; a fucose concentration from about 0 mM to about 60 mM; and a cultivation temperature from about 29°C to about 39°C, wherein the cell culture duration is from about 0 days to about 150 days.

In certain embodimentsexamples, the step of modulating the glycosylation pattern of the glycoprotein of interest comprises: modulating the Na⁺ concentration of about 0 nM to about 300 nM and any of the following parameters, either alone or in any combination, in

the cell culture medium, and/or in the cell culture environment: an osmolality from about 250 mOsm/kg to about 550 mOsm/kg; a galactose concentration from about 0 mM to about 60 mM; a fucose concentration from about 0 mM to about 60 mM; and a cultivation temperature from about 29°C to about 39°C, wherein the Na⁺ concentration from about 0 mM to about 300 mM.

In certain embodimentsexamples, the step of modulating the glycosylation pattern of the glycoprotein of interest comprises: modulating the Na⁺ concentration and the pCO₂ from about 10 mmHg to about 250 mmHg.

In certain embodimentsexamples, the step of modulating the glycosylation pattern of the glycoprotein of interest comprises: modulating the osmolality and any of the following parameters, either alone or in any combination, in the cell culture medium, and/or in the cell culture environment: a galactose concentration from about 0 mM to about 60 mM; a fucose concentration from about 0 mM to about 60 mM; and a cultivation temperature from about 29°C to about 39°C, wherein the osmolality is from about 250 mOsm/kg to about 550 mOsm/kg.

In certain embodimentsexamples, the step of modulating the glycosylation pattern of the glycoprotein of interest comprises: modulating the Mn concentration from about 1 nM to about 30000 nM under a low pCO₂ condition, or modulating the Mn concentration from about 1 nM to about 20000 nM under a high pCO₂ condition, modulating the Na⁺ concentration from about 0 mM to about 300 mM, and modulating the duration of the pre-inoculation cell culture media hold from about 0 hrs to about 120 hrs.

In certain embodimentsexamples, modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the osmolality from about 250 mOsm/kg to about 550 mOsm/kg and the pCO₂ from about 10 mmHg to about 250 mmHg.

In certain embodimentsexamples, the modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating: the cultivation temperature from about 29°C to about 39°C, and, the galactose concentration from about 0 mM to about 60 mM; and/or the fucose concentration from about 0 mM to about 60 mM.

In certain embodimentsexamples, modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the pCO₂ from about 10 mmHg to about 250 mmHg and the fucose concentration from about 0 mM to about 60 mM.

In certain embodimentsexamples, modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the fucose concentration from about 0 mM to about 60 mM and the cultivation temperature from about 29°C to about 39°C.

5 In certain embodimentsexamples, the modulation of the glycosylation pattern of the glycoprotein of interest comprises: modulating a pCO₂ concentration and any of the following parameters, either alone or in any combination, in the cell culture medium, and/or in the cell culture environment: a Mn concentration from about 1 nM to about 20000 nM in a high partial pressure CO₂ (pCO₂) condition; a Mn concentration from about 1 nM to about 30000 nM in a low pCO₂ condition; a pre-inoculation cell culture media hold
10 duration from about 0 hrs to about 120 hrs at a temperature of about 25°C to 39°C; a cell culture duration from about 0 days to about 150 days; a Na⁺ concentration from about 0 mM to about 300 mM; an osmolality from about 250 mOsm/kg to about 550 mOsm/kg; a galactose concentration from about 0 mM to about 60 mM; a fucose concentration from about 0 mM to about 60 mM; and a cultivation temperature from about 29°C to about
15 39°C, wherein the pCO₂ concentration is from about 10 mmHg to about 250 mmHg.

In certain embodimentsexamples, the Mn concentration is from about 1 nM to about 20000 nM in a high pCO₂ culture; from about 1 nM to about 10000 nM, from about 1 nM to about 5000 nM, from about 1 nM to about 4000 nM, from about 1 nM to about 3000 nM, from about 1 nM to about 2000 nM, from about 1 nM to about 1000 nM in a
20 high pCO₂ culture; from about 1 nM to about 500 nM, from about 1 nM to about 100 nM, from about 1 nM to about 50 nM, from about 1 nM to about 20 nM, from about 20 nM to about 2000 nM, from about 20 nM to about 3000 nM, from about 20 nM to about 10000 nM, from about 20 nM to about 20,000 nM, from about 20 nM to about 300 nM, about 30 nM to about 110 nM in a high pCO₂ culture.

25 In certain embodimentsexamples, the Mn concentration is about 1 nM to about 30000 nM in a low pCO₂ culture; from about 1 nM to about 20000 nM; from about 1 nM to about 10000 nM, from about 1 nM to about 5000 nM, from about 1 nM to about 4000 nM, from about 1 nM to about 3000 nM, from about 1 nM to about 2000 nM, from about 1 nM to about 1000 nM; from about 1 nM to about 500 nM, from about 1 nM to about 100
30 nM, from about 1 nM to about 50 nM, from about 1 nM to about 20 nM, from about 20 nM to about 100 nM, about 20 nM to about 300 nM, from about 20 nM to about 500 nM, from about 20 nM to about 1000 nM, from about 20 nM to about 2000 nM, from about 20 nM

to about 3000 nM, from about 20 nM to about 5000 nM, from about 20 nM to about 10000 nM, from about 20 nM to about 20000 nM, or about 30 nM to about 110 nM in a low pCO₂ culture.

5 In certain embodimentsexamples, 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.

10 In certain embodimentsexamples, 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. In certain embodimentsexamples, 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). In certain embodimentsexamples, the filter includes but is not limited to: a depth filter, a column, a membrane and a disc. In certain
15 embodimentsexamples, the filter material includes but is not limited to: diatomaceous earth, hollow fibers or a resin.

In certain embodimentsexamples, the cell culture medium is a basal medium, a reconstituted medium, a feed medium, a hydrolysate, a supplement, serum or an additive.

20 In certain embodimentsexamples, the cell culture medium is supplemented during the production stage of the cell culture.

In certain embodimentsexamples, the cell culture medium is supplemented prior to the production stage of the cell culture.

25 In certain embodimentsexamples, the cell culture medium comprises one or more of: Mn, fucose, galactose and/or Na⁺, and wherein the supplementation is based on a pre-defined schedule or criteria.

In certain embodimentsexamples, the one or more of the Mn, fucose, galactose and Na⁺ 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.

30 In certain embodimentsexamples, the cell culture medium consists essentially of one or more of: i) Mn; ii) fucose; iii) galactose; and/or iv) Na⁺.

In certain [embodimentsexamples](#), the modulation of the Mn concentration comprises employing a cell culture media pH of about 6.1 to about 7.3; or about 6.3 to about 7.3 prior to High Temperature Short Time (HTST) heat treatment.

5 In certain [embodimentsexamples](#), modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the pCO₂.

10 In certain [embodimentsexamples](#), the cell culture or cell culture media is in a bioreactor and where modulation of pCO₂ is achieved by modulating: the bioreactor working volume; the bioreactor gas sparging strategy; the bioreactor agitation strategy; the bioreactor media exchange strategy, the bioreactor perfusion strategy, the bioreactor feed strategy, or an any combination thereof.

In certain [embodimentsexamples](#), the pCO₂ is modulation comprises establishing a high pCO₂ culture. In certain [embodimentsexamples](#), the pCO₂ is about 20 mmHg to about 250 mmHg; about 20 mmHg to about 250 mmHg; about 20 mmHg to about 150 mmHg; or about 30 mmHg to about 250 mmHg.

15 In certain [embodimentsexamples](#), the pCO₂ is modulation comprises establishing a low pCO₂ culture. In certain [embodimentsexamples](#), the pCO₂ is about 10 mmHg to about 100 mmHg; 10 mmHg to about 80 mmHg; about 20 mmHg to about 70 mmHg; or about 30 mmHg to about 60 mmHg.

20 In certain [embodimentsexamples](#), the pCO₂ modulation occurs at day 0 of the culture.

In certain [embodimentsexamples](#), the pCO₂ modulation occurs for: about the majority of the cell culture; about the first 5 days; about the first 7 days; or about the first 10 days.

25 In certain [embodimentsexamples](#), the pCO₂ modulation occurs for: about the majority of the production culture; about the first 5 days; about the first 7 days; or about the first 10 days.

30 In certain [embodimentsexamples](#), modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the duration of the pre-inoculation cell culture media hold, wherein the duration of the pre-inoculation cell culture media hold is about 0 hrs to about 120 hrs; about 0 hrs to about 72 hrs; about 0 hrs to about 48 hrs; or about 0 hrs to about 24 hrs.

In certain [embodimentsexamples](#), the temperature of the media during the pre-inoculation cell culture media hold is about 25°C to about 39°C; about 30°C to about 39°C; about 35°C to about 39°C; or about 36°C to about 39°C.

In certain [embodiments](#)[examples](#), 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 about 0 days to about 150 days; about 0 days to about 15 days; about 0 days to about 12 days; 0 days to about 7 days; or about 0 days to about 5 days.

In certain [embodiments](#)[examples](#), modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the Na⁺ concentration, wherein the Na⁺ concentration is about 0 mM to about 300 mM; is about 20 mM to about 20 mM; about 30 mM to about 150 mM; or about 40 mM to about 130 mM.

In certain [embodiments](#)[examples](#), the modulation of the Na⁺ concentration comprises supplementing the cell culture with Na compounds including but not limited to: Na₂CO₃, NaHCO₃, NaOH, NaCl, or combinations thereof.

In certain [embodiments](#)[examples](#), modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the osmolality, wherein the osmolality of is about 250 mOsm/kg to about 550 mOsm/kg; about 300 mOsm/kg to about 450 mOsm/kg; or about 325 mOsm/kg to about 425 mOsm/kg. In certain [embodiments](#)[examples](#), the modulation of the osmolality comprises supplementing the cell culture with an osmolality-modulating media component. In certain [embodiments](#)[examples](#), the osmolality-modulating media component is NaCl, KCl, sorbitol, an osmoprotectant, or combinations thereof. In certain [embodiments](#)[examples](#), the osmolality-modulating media component 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.

In certain [embodiments](#)[examples](#), modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the galactose concentration, wherein the galactose concentration is about 0 mM to about 60 mM or about 0 mM to about 50 mM.

In certain [embodiments](#)[examples](#), modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the fucose concentration, wherein the fucose concentration is about 0 mM to about 60 mM; 0 mM to about 40 mM; about 0 mM to about 20 mM; or about 0 mM to about 10 mM.

In certain [embodiments](#)[examples](#), modulation of the glycosylation pattern of the glycoprotein of interest comprises modulating the cell culture temperature, wherein the

cell culture temperature is about 29°C to about 39°C; about 30°C to about 39°C; about 31°C to about 38°C; or about 34°C to about 38°C.

In certain embodimentsexamples, the cell culture temperature is modulated during the production stage of the cell culture.

5 In certain embodimentsexamples, the cell culture temperature is modulated prior the production stage of the cell culture.

In certain embodimentsexamples, the cell culture temperature is modulated based on a pre-defined schedule or criteria.

10 In certain embodimentsexamples, the cell culture comprises eukaryotic cells. In certain embodimentsexamples, the eukaryotic cells are insect, avian, fungal, plant or mammalian cells. In certain embodimentsexamples, the fungal cells are yeast, *Pichia* or any filamentous fungal cells. In certain embodimentsexamples, the yeast cells are *S. cerevisiae* cells. In certain embodimentsexamples, the mammalian cells are CHO cells.

15 In certain embodimentsexamples, the cell culture is in a bioreactor including but not limited to: a single use technology (SUT) bag or bioreactor; a WAVE bioreactor; a stainless steel bioreactor; a flask; a tube and a chamber.

20 In certain embodimentsexamples, the volume of the cell culture is from 1 mL to 35,000 L. In certain embodimentsexamples, the volume of the cell culture is from 1 mL to 10ml, from 1 mL to 50ml, from 1 mL to 100ml, from 1 mL to 200ml, from 1 mL to 300ml, from 1 mL to 500ml, from 1 mL to 1000ml, from 1 mL to 2000ml, from 1 mL to 3000ml, from 1 mL to 4000ml, from 1 mL to 5000ml, from 1 mL to 1L, from 1 mL to 2L, from 1 mL to 3L, from 1 mL to 4L, from 1 mL to 5L, from 1 mL to 6L, from 1 mL to 10L, from 1 mL to 20L, from 1 mL to 30L, from 1 mL to 40L, from 1 mL to 50L, from 1 mL to 60L, from 1 mL to 70L, from 1 mL to 100L, from 1 mL to 200L, from 1 mL to 300L, 25 from 1 mL to 400L, from 1 mL to 500L, from 1 mL to 1000L, from 1 mL to 2000L, from 1 mL to 3000L, from 1 mL to 4000L, from 1 mL to 5000L, from 1 mL to 10,000L, from 1 mL to 20,000L, from 1 mL to 30,000L, from 1 mL to 30,000L, from 1 mL to 35,000 L.

30 In certain embodimentsexamples, the present disclosure is directed to methods to prepare a cell culture media, a feed media, a hydrolysate, or an additive comprising one or more step(s) of modulating: the Mn concentration in a high partial pressure CO₂ (pCO₂) culture from about 1 nM to about 20000 nM; the Mn concentration in a low pCO₂ culture from about 1 nM to about 30000 nM; the pCO₂ from about 10 mmHg to about 250 mmHg; the

pre-inoculation cell culture media hold duration from about 0 hrs to about 120 hrs; the cell culture duration from about 0 days to about 150 days; the Na⁺ concentration from about 0 mM to about 300 mM; the osmolality from about 250 mOsm/kg to about 550 mOsm/kg; the galactose concentration from about 0 mM to about 60 mM; the fucose concentration
5 from about 0 mM to about 60 mM; and the cultivation temperature from about 29°C to about 39°C; wherein the cell culture media, feed media, hydrolysate, or additive modulates the glycosylation pattern of a glycoprotein of interest.

In certain embodimentsexamples, the methods involve modulating the pCO₂ from about 10 mmHg to about 250 mmHg, the Na⁺ concentration from about 0 mM to
10 about 300 mM, and the duration of the pre-inoculation cell culture media hold from about 0 hrs to about 120 hrs.

In certain embodimentsexamples, the methods involve modulating the Mn concentration from about 1 nM to about 30000 nM, the pCO₂ from about 10 mmHg to about 250 mmHg, and the Na⁺ concentration from about 0 mM to about 300 mM.

15 In certain embodimentsexamples, the methods involve modulating the Mn concentration from about 1 nM to about 30000 nM, the pCO₂ from about 10 mmHg to about 250 mmHg, the Na⁺ concentration from about 0 mM to about 300 mM, and the duration of the pre-inoculation cell culture media hold from about 0 hrs to about 72 hrs.

In certain embodimentsexamples, the methods involve modulating the pCO₂
20 from about 10 mmHg to about 250 mmHg and the Na⁺ concentration from about 0 mM to about 300 mM.

In certain embodimentsexamples, the methods involve modulating the osmolality from about 250 mOsm/kg to about 550 mOsm/kg and the pCO₂ from about 10 mmHg to about 250 mmHg.

25 In certain embodimentsexamples, the methods involve modulating the pCO₂ from about 10 mmHg to about 250 mmHg, the Mn concentration from about 1 nM to about 30000 nM, the duration of the cell culture from about 0 days to about 150 days, and the duration of the pre-inoculation cell culture media hold from about 0 hrs to about 120 hrs

In certain embodimentsexamples, the methods involve modulating the Mn
30 concentration from about 1 nM to about 30000 nM and the galactose concentration from about 0 mM to about 60 mM.

In certain [embodimentsexamples](#), the methods involve modulating the fucose concentration from about 0 mM to about 60 mM and the Mn concentration from about 1 nM to about 30000 nM.

5 In certain [embodimentsexamples](#), the methods involve modulating the fucose concentration from about 0 mM to about 60 mM and the pCO₂ from about 10 mmHg to about 250 mmHg.

In certain [embodimentsexamples](#), the methods involve modulating the fucose concentration from about 0 mM to about 60 mM, the Mn concentration from about 1 nM to about 30000 nM, and the pCO₂ from about 10 mmHg to about 250 mmHg.

10 In certain [embodimentsexamples](#), the methods involve modulating the fucose concentration from about 0 mM to about 60 mM and the cell culture temperature is about 29°C to about 39°C.

In certain [embodimentsexamples](#), the methods involve modulating the fucose concentration from about 0 mM to about 60 mM and the duration of the cell culture from
15 about 0 days to about 150 days.

In certain [embodimentsexamples](#), the Mn concentration is about 1 nM to about 20000 nM in a high pCO₂ culture; about 1 nM to about 1000 nM in a high pCO₂ culture; about 20 nM to about 300 nM in a high pCO₂ culture; or about 30 nM to about 110 nM in a high pCO₂ culture.

20 In certain [embodimentsexamples](#), the Mn concentration is about 1 nM to about 30000 nM in a low pCO₂ culture; about 1 nM to about 3000 nM in a low pCO₂ culture; about 20 nM to about 300 nM in a low pCO₂ culture; or about 30 nM to about 110 nM in a low pCO₂ culture.

In certain [embodimentsexamples](#), modulation of the Mn concentration
25 comprises determining the Mn content in cell culture raw materials and selecting raw material lots to modulate the Mn concentration.

In certain [embodimentsexamples](#), 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
30 during cell culture; or a combination of (i) and (ii) to modulate the Mn concentration.

In certain [embodimentsexamples](#), 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). In certain embodimentsexamples, the filter includes but is not limited to: a depth filter, a column, a membrane and a disc. In certain embodimentsexamples, the filter material includes but is not limited to: diatomaceous earth, hollow fibers or a resin.

5 In certain embodimentsexamples, the modulation of the Mn concentration comprises employing a cell culture media pH of about 6.1 to about 7.3; or about 6.3 to about 7.3 prior to HTST treatment.

In certain embodimentsexamples, the pCO₂ is modulated. In certain embodimentsexamples, the cell culture media is in a bioreactor and where modulation of
10 pCO₂ is achieved by modulating: the bioreactor working volume; the bioreactor gas sparging strategy; the bioreactor agitation strategy; the bioreactor feed strategy; the bioreactor perfusion strategy; the bioreactor media exchange strategy; or any combination thereof. In certain embodimentsexamples, the pCO₂ modulation comprises establishing a high pCO₂ culture. In certain embodimentsexamples, the pCO₂ is about 20 mmHg to about
15 250 mmHg; about 20 mmHg to about 250 mmHg; about 20 mmHg to about 150 mmHg; or about 30 mmHg to about 150 mmHg.

In certain embodimentsexamples, the pCO₂ is modulation comprises establishing a low pCO₂ culture. In certain embodimentsexamples, the pCO₂ is about 10 mmHg to about 100 mmHg; 10 mmHg to about 80 mmHg; about 20 mmHg to about 70
20 mmHg; or about 30 mmHg to about 60 mmHg. In certain embodimentsexamples, the pCO₂ modulation occurs at day 0 of the culture. In certain embodimentsexamples, the pCO₂ modulation occurs for: about the majority of the cell culture; about the first 5 days; about the first 7 days; or about the first 10 days. In certain embodimentsexamples, the pCO₂ modulation occurs for: about the majority of the production culture; about the first
25 5 days; about the first 7 days; or about the first 10 days.

In certain embodimentsexamples, the duration of the pre-inoculation cell culture media hold is about 0 hrs to about 120 hrs; 0 hrs to about 72 hrs; about 0 hrs to about 48 hrs; or about 0 hrs to about 24 hrs. In certain embodimentsexamples, the temperature of the media during the pre-inoculation cell culture media hold is about 25°C
30 to about 39°C; about 30°C to about 39°C; about 35°C to about 39°C; or about 36°C to about 39°C.

In certain embodimentsexamples, the duration of the cell culture is about 0 days to about 150 days; about 0 days to about 15 days; about 0 days to about 12 days; 0 days to about 7 days; or about 0 days to about 5 days.

In certain embodimentsexamples, the Na⁺ concentration is about 0 mM to about 300 mM; is about 20 mM to about 200 mM; about 30 mM to about 150 mM; or about 40 mM to about 130 mM. In certain embodimentsexamples, the modulation of the Na⁺ concentration comprises supplementing the cell culture with Na compounds including but not limited to: Na₂CO₃, NaHCO₃, NaOH, NaCl, or combinations thereof.

In certain embodimentsexamples, the osmolality of is about 250 mOsm/kg to about 550 mOsm/kg; about 300 mOsm/kg to about 450 mOsm/kg; or about 325 mOsm/kg to about 425 mOsm/kg. In certain embodimentsexamples, the modulation of the osmolality comprises supplementing the cell culture with an osmolality-modulating media component. In certain embodimentsexamples, the osmolality-modulating media component is NaCl, KCl, sorbitol, an osmoprotectant, or combinations thereof.

In certain embodimentsexamples, the galactose concentration is about 0 mM to about 60 mM or about 0 mM to about 50 mM.

In certain embodimentsexamples, the fucose concentration is about 0 mM to about 60 mM; 0 mM to about 40 mM; about 0 mM to about 20 mM; or about 0 mM to about 10 mM.

In certain embodimentsexamples, the cell culture temperature is about 29°C to about 39°C; about 30°C to about 39°C; about 31°C to about 38°C; or about 34°C to about 38°C.

In certain embodimentsexamples, the present disclosure is directed to a eukaryotic cell fermentation process for the production of a recombinant protein. In certain embodimentsexamples, 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 embodimentsexamples, the antibody is a chimeric, a humanized or a human antibody. In certain embodimentsexamples, the antibody is an anti-CD20 antibody. In certain embodimentsexamples, the anti-CD20 antibody is ocrelizumab. In certain embodimentsexamples, 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%; 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%. In certain embodimentsexamples, the eukaryotic cell is an insect, avian, fungal, plant or mammalian cell. In certain embodimentsexamples, the fungal cells are yeast, *Pichia* or any filamentous fungal cells. In certain embodimentsexamples, the yeast cells are *S. cerevisiae* cells. In certain embodimentsexamples, the mammalian cells are CHO cells.

In certain embodimentsexamples, the present disclosure is directed to a cell culture composition comprising, a host cell engineered to express a glycoprotein of interest; and a cell culture and/or cell culture media modulated to target one or more predetermined parameter selected from: the Mn concentration in a high partial pressure CO₂ (pCO₂) culture from about 1 nM to about 20000 nM; the Mn concentration in a low pCO₂ culture from about 1 nM to about 30000 nM; the pCO₂ from about 10 mmHg to about 250 mmHg; the pre-inoculation cell culture media hold duration from about 0 hrs to about 120 hrs; the cell culture duration from about 0 days to about 150 days; the Na⁺ concentration from about 0 mM to about 300 mM; the osmolality from about 250 mOsm/kg to about 550 mOsm/kg; the galactose concentration from about 0 mM to about 60 mM; the fucose concentration from about 0 mM to about 60 mM; and the cultivation temperature from about 29°C to about 39°C.

In certain embodimentsexamples, the Mn concentration is from about 1 nM to about 30000 nM and the duration of the pre-inoculation cell culture media hold is from about 0 hrs to about 120 hrs.

In certain embodimentsexamples, the pCO₂ is from about 10 mmHg to about 250 mmHg, the Na⁺ concentration from about 0 mM to about 300 mM, and the duration of the pre-inoculation cell culture media hold is from about 0 hrs to about 120 hrs.

In certain embodimentsexamples, the Mn concentration is from about 1 nM to about 30000 nM, the pCO₂ is from about 10 mmHg to about 250 mmHg, and the Na⁺ concentration is from about 0 mM to about 300 mM.

In certain embodimentsexamples, the Mn concentration is from about 1 nM to about 30000 nM, the pCO₂ is from about 10 mmHg to about 250 mmHg, the Na⁺ concentration is from about 0 mM to about 300 mM, and the duration of the pre-inoculation cell culture media hold is from about 0 hrs to about 120 hrs.

In certain embodimentsexamples, the pCO₂ is from about 10 mmHg to about 250 mmHg and the Na⁺ concentration is from about 0 mM to about 300 mM.

In certain [embodimentsexamples](#), the osmolality is from about 250 mOsm/kg to about 550 mOsm/kg and the pCO₂ is from about 10 mmHg to about 250 mmHg.

In certain [embodimentsexamples](#), the pCO₂ is from about 10 mmHg to about 250 mmHg, the Mn concentration is from about 1 nM to about 30000 nM, the duration of the cell culture is from about 0 days to about 150 days, and the duration of the pre-inoculation cell culture media hold is from about 0 hrs to about 120 hrs

In certain [embodimentsexamples](#), the Mn concentration is from about 1 nM to about 30000 nM and the galactose concentration is from about 0 mM to about 60 mM. In certain [embodimentsexamples](#), the fucose concentration is from about 0 mM to about 60 mM and the Mn concentration is from about 1 nM to about 30000 nM.

In certain [embodimentsexamples](#), the fucose concentration is from about 0 mM to about 60 mM and the pCO₂ is from about 10 mmHg to about 250 mmHg. In certain [embodimentsexamples](#), the fucose concentration is from about 0 mM to about 60 mM, the Mn concentration is from about 1 nM to about 30000 nM, and the pCO₂ is from about 10 mmHg to about 250 mmHg. In certain [embodimentsexamples](#), the fucose concentration is from about 0 mM to about 60 mM and the cell culture temperature is about 29°C to about 39°C. In certain [embodimentsexamples](#), the fucose concentration is from about 0 mM to about 60 mM and the duration of the cell culture is from about 0 days to about 150 days.

In certain [embodimentsexamples](#), the present disclosure is directed to methods for producing a glycoprotein of interest in a cell culture, comprising: subjecting a cell culture medium suitable for cultivating a eukaryotic cell to the method according to any one of [embodimentsexamples](#) disclosed herein, inoculating the modulated cell culture medium with the eukaryotic cell that expresses the recombinant protein; cultivating the eukaryotic cell so that the recombinant protein is expressed.

In certain [embodimentsexamples](#), the present disclosure is directed to methods of modulating the glycosylation of a glycoprotein of interest, the method comprising: assaying cell culture media to determine if the manganese concentration of the cell culture media falls within a targeted range; and culture a host cell engineered to express the glycoprotein of interest in the cell culture media falling within the targeted range; wherein the glycosylation of glycoproteins of interest is modulated as compared to the glycosylation of glycoproteins of interest expressed by the host cell in culture media falling outside the targeted range of manganese concentrations.

In certain embodimentsexamples, the present disclosure is directed to compositions comprising a glycoprotein of interest, wherein the preparation comprises: a cell culture media assayed to determine if the manganese concentration of the cell culture media falls within a targeted range; a host cell engineered to express a glycoprotein of interest; and the glycoprotein of interest.

In certain embodimentsexamples, the present disclosure is directed to methods of modulating the glycosylation of a glycoprotein of interest, the method comprising: supplementing a cell culture media employed in culturing host cells expressing the glycoprotein of interest with between about 10nM and about 2000nM manganese under high CO₂ conditions; or supplementing the cell culture media employed in culturing a host cell expressing the glycoprotein of interest with between about 10nM and about 3000nM manganese under low CO₂ conditions; wherein the glycosylation of glycoproteins of interest is modulated as compared to the glycosylation of glycoproteins of interest expressed by the host cell in culture media that has not been so supplemented.

In certain embodimentsexamples, the present disclosure is directed to cell culture compositions comprising, a cell culture media supplemented with: between about 10nM and about 2000nM manganese under high CO₂ conditions; or between about 10nM and about 3000nM manganese under low CO₂ conditions; and a host cell engineered to express a glycoprotein of interest.

In certain embodimentsexamples, the present disclosure is directed to compositions comprising a glycoprotein of interest, wherein the preparation comprises: a manganese supplemented cell culture media wherein the culture is supplemented with between about 10nM and about 2000nM manganese under high CO₂ conditions; or between about 10nM and about 3000nM manganese under low CO₂ conditions; a host cell engineered to express the glycoprotein of interest; and the glycoprotein of interest.

In certain embodimentsexamples, the method of modulating the glycosylation of a glycoprotein of interest comprises assaying cell culture media and/or cell cultures to determine if the manganese concentration of the cell culture media and/or cell cultures falls within a targeted range and culture a host cell engineered to express the glycoprotein of interest in the cell culture media falling within the targeted range. In certain embodimentsexamples, the glycosylation of glycoproteins of interest is modulated as compared to

the glycosylation of glycoproteins of interest expressed by the host cell in culture media and/or cell cultures falling outside the targeted range of manganese concentrations. In some [embodimentsexamples](#), the manganese concentration target range is between 20 nM and 200 nM. In non-limiting [embodimentsexamples](#), the manganese concentration target range is between about 30 nM and about 110 nM.

In certain [embodimentsexamples](#), the disclosed glycoprotein of interest is an antibody. The antibody can be a chimeric antibody, a humanized antibody, or a human antibody. In non-limiting [embodimentsexamples](#), the antibody is ocrelizumab.

In certain [embodimentsexamples](#), the disclosed host cell is a mammalian cell. The host cell can be a Chinese Hamster Ovary (CHO) cell.

In certain [embodimentsexamples](#), the disclosed assaying of the cell culture media comprises assaying the manganese concentration of a component of the cell culture media. In certain [embodimentsexamples](#), the disclosed assaying of the cell culture comprises assaying the manganese concentration of a component of the cell culture. The component of the cell culture media is a hydrolysate or a serum. The component of the cell culture media can also be a complex blend of multiple components.

In certain [embodimentsexamples](#), the glycosylation is modulated to achieve an increased afucosylation (e.g., G0-F (afucosylated G0)), while decreasing agalactosylation (e.g., G0 (fucosylated, agalactosylated G0)). In certain [embodimentsexamples](#), the glycosylation is modulated to achieve a decreased afucosylation (e.g., G0-F), while increasing agalactosylation (e.g., G0).

In certain [embodimentsexamples](#), glycosylation is modulated to achieve an increased or decreased afucosylation (e.g., G0-F) without impacting agalactosylation (e.g., G0). In certain [embodimentsexamples](#), glycosylation is modulated to achieve an increased or decreased agalactosylation (e.g., G0) without impacting afucosylation (e.g., G0-F).

In certain [embodimentsexamples](#), the subject matter disclosed herein is directed to a cell culture composition comprising, a cell culture media and/or cell cultures assayed to determine if the manganese concentration of the cell culture media and/or cell cultures falls within a targeted range; and a host cell engineered to express a glycoprotein of interest. In certain [embodimentsexamples](#), the manganese concentration is controlled through the selection or avoidance of raw materials that are in contact with culture media and/or cell cultures and can leach manganese (e.g., depth and/or media filters, media preparation

and/or hold vessels, and bioreactors). In certain embodimentsexamples, the cell culture composition further comprises the glycoprotein of interest. In certain embodimentsexamples, the glycoprotein of interest is an antibody. In certain embodimentsexamples, the antibody is a chimeric antibody, a humanized antibody, or a human antibody. In certain embodimentsexamples, the antibody is ocrelizumab. In certain embodimentsexamples, the host cell is a mammalian cell, e.g., a CHO cell.

In certain embodimentsexamples, the subject matter disclosed herein is directed to a preparation comprising a glycoprotein of interest, wherein the preparation comprises a cell culture media assayed to determine if the manganese concentration of the cell culture media falls within a targeted range; a host cell engineered to express a glycoprotein of interest; and the glycoprotein of interest. In certain embodimentsexamples, the manganese concentration is controlled through the selection of raw materials that contain manganese at the desired levels. In certain embodimentsexamples, the glycoprotein of interest is an antibody. In certain embodimentsexamples, the antibody is a chimeric antibody, a humanized antibody, or a human antibody. In certain embodimentsexamples, the antibody is ocrelizumab. In certain embodimentsexamples, the host cell is a mammalian cell, e.g., a CHO cell.

In certain embodimentsexamples, the subject matter disclosed herein is directed to a method of modulating the glycosylation of a glycoprotein of interest, the method comprising supplementing a cell culture media employed in culturing host cells expressing the glycoprotein of interest with between about 10 nM and about 2000 nM manganese under high CO₂ conditions; or supplementing the cell culture supplementing the cell culture media employed in culturing a host cell expressing the glycoprotein of interest with between about 10 nM and about 3000 nM manganese under low CO₂ conditions; wherein the glycosylation of glycoproteins of interest is modulated as compared to the glycosylation of glycoproteins of interest expressed by the host cell in culture media that has not been so supplemented. In certain embodimentsexamples, the manganese is supplemented directly to the cell cultures. In certain embodimentsexamples, the glycoprotein of interest is an antibody. In certain embodimentsexamples, the antibody is a chimeric antibody, a humanized antibody, or a human antibody. In certain embodimentsexamples, the antibody is ocrelizumab. In certain embodimentsexamples, the host cell is a mammalian cell, e.g., a CHO cell. In certain embodimentsexamples, the glycosylation is modulated to achieve an increased afucosylation (e.g., G0-F (afucosylated G0)), while decreasing agalactosylation (e.g., G0 (fucosylated G0)). In certain embodimentsexamples, the glycosylation is modulated to achieve a decreased

afucosylation (e.g., G0-F), while increasing agalactosylation (e.g., G0).

In certain embodimentsexamples, the subject matter disclosed herein is directed to a cell culture composition comprising, a cell culture media and/or cell culture supplemented with: between about 10nM and about 2000nM manganese under high CO₂ conditions; or
5 between about 10nM and about 3000nM manganese under low CO₂ conditions; and a host cell engineered to express a glycoprotein of interest. In certain embodimentsexamples, the glycoprotein of interest is an antibody. In certain embodimentsexamples, the antibody is a chimeric antibody, a humanized antibody, or a human antibody. In certain embodimentsexamples, the antibody is ocrelizumab. In certain embodimentsexamples, the
10 host cell is a mammalian cell, e.g., a CHO cell.

In certain embodimentsexamples, the subject matter disclosed herein is directed to a composition comprising a glycoprotein of interest, wherein the preparation comprises a manganese supplemented cell culture media wherein the culture is supplemented with between
15 about 10nM and about 2000nM manganese under high CO₂ conditions; or between about 10nM and about 3000nM manganese under low CO₂ conditions; a host cell engineered to express the glycoprotein of interest; and the glycoprotein of interest. In certain embodimentsexamples, the manganese is supplemented by using raw materials that leach manganese during their contact with culture media and/or cell cultures (e.g., depth and/or media filters, media preparation and/or hold vessels, and bioreactors). In certain
20 embodimentsexamples, the glycoprotein of interest is an antibody. In certain embodimentsexamples, the antibody is a chimeric antibody, a humanized antibody, or a human antibody. In certain embodimentsexamples, the antibody is ocrelizumab. In certain embodimentsexamples, the host cell is a mammalian cell, e.g., a CHO cell.

In certain embodimentsexamples, the subject matter disclosed herein is directed to
25 a method of modulating the glycosylation of a glycoprotein of interest, the method comprising exposing cell culture media comprising a pH target of about 6.10 to about 7.25 to high temperature short time (HTST) heat treatment; and culturing a host cell expressing the glycoprotein of interest in the cell culture media; wherein the glycosylation of the glycoproteins of interest is modulated as compared to the glycosylation of the glycoproteins
30 of interest expressed by the host cell in culture media where the pre-HTST heat treatment pH target is greater than pH 7.25. In certain embodimentsexamples, the glycoprotein of interest is an antibody. In certain embodimentsexamples, the antibody is a chimeric antibody, a

humanized antibody, or a human antibody. In certain embodimentsexamples, the antibody is ocrelizumab. In certain embodimentsexamples, the host cell is a mammalian cell, e.g., a CHO cell. In certain embodimentsexamples, the glycosylation is modulated to achieve an increased G0-F (afucosylated G0), while decreasing G0 (fucosylated G0).

5 In certain embodimentsexamples, the subject matter disclosed herein is directed to a method of modulating the Mn level in the cell culture media and/or cell culture comprised of employing a cell culture media pH of about 6.1 to about 7.3 prior to High Temperature Short Time (HTST) heat treatment; and culturing a host cell expressing the glycoprotein of interest in the cell culture media. In certain embodimentsexamples, the glycoprotein of interest
10 is an antibody. In certain embodimentsexamples, the antibody is a chimeric antibody, a humanized antibody, or a human antibody. In certain embodimentsexamples, the antibody is ocrelizumab. In certain embodimentsexamples, the host cell is a mammalian cell, e.g., a CHO cell. In certain embodimentsexamples, the glycosylation is modulated to achieve an increased G0-F (afucosylated G0), while decreasing G0 (fucosylated G0).

15 In certain embodimentsexamples, the subject matter disclosed herein is directed to a cell culture composition comprising, a cell culture media comprising a pH target of about 6.30 to about 7.25 exposed to a HTST heat treatment; and a host cell engineered to express a glycoprotein of interest. In certain embodimentsexamples, the glycoprotein of interest is an antibody. In certain embodimentsexamples, the antibody is a chimeric antibody, a humanized
20 antibody, or a human antibody. In certain embodimentsexamples, the antibody is ocrelizumab. In certain embodimentsexamples, the host cell is a mammalian cell, e.g., a CHO cell.

In certain embodimentsexamples, the subject matter disclosed herein is directed to a cell culture composition comprising, a cell culture media comprising a pH target of about 6.3 to about 7.3 prior to exposure to a HTST heat treatment; and a host cell engineered to
25 express a glycoprotein of interest. In certain embodimentsexamples, the glycoprotein of interest is an antibody. In certain embodimentsexamples, the antibody is a chimeric antibody, a humanized antibody, or a human antibody. In certain embodimentsexamples, the antibody is ocrelizumab. In certain embodimentsexamples, the host cell is a mammalian cell, e.g., a CHO cell.

30 In certain embodimentsexamples, the subject matter disclosed herein is directed to a composition comprising a glycoprotein of interest, wherein the preparation comprises a cell culture media comprising a pH target of about 6.10 to about 7.25 exposed to HTST heat treatment; a host cell engineered to express a glycoprotein of interest; and the

glycoprotein of interest. In certain embodimentsexamples, the glycoprotein of interest is an antibody. In certain embodimentsexamples, the antibody is a chimeric antibody, a humanized antibody, or a human antibody. In certain embodimentsexamples, the antibody is ocrelizumab. In certain embodimentsexamples, the host cell is a mammalian cell, e.g., a CHO cell.

5 In certain embodimentsexamples, the subject matter disclosed herein is directed to a composition comprising a glycoprotein of interest, wherein the preparation comprises a cell culture media comprising a pH target of about 6.1 to about 7.3 prior to exposure to HTST heat treatment; a host cell engineered to express a glycoprotein of interest; and the glycoprotein of interest.

10 In certain embodimentsexamples, the subject matter disclosed herein is directed to a method of modulating the glycosylation of a glycoprotein of interest, the method comprising: culturing a host cell expressing the glycoprotein of interest in a cell culture media where the cell culture is supplemented with higher or lower levels of manganese, galactose, and/or fucose (or no supplementation), exposed to high or low pCO₂, the cell culture is exposed to an
15 extended or shortened media hold time and/or culture duration, the culture is maintained at higher or lower cultivation temperature, maintained at higher or lower osmolality, and/or the cell culture comprises an increased or decreased Na⁺ concentration, and/or any combinations thereof; wherein the glycosylation of the glycoproteins of interest is modulated as compared to the fucosylation and/or galactosylation of a preparation of glycoproteins of interest
20 expressed by the host cell in culture media exposed to low pCO₂, a shortened media hold time, and/or a reduced Na⁺ concentration. In certain embodimentsexamples, the glycoprotein of interest is an antibody. In certain embodimentsexamples, the antibody is a chimeric antibody, a humanized antibody, or a human antibody. In certain embodimentsexamples, the antibody is ocrelizumab. In certain embodimentsexamples, the host cell is a mammalian cell, e.g., a
25 CHO cell. In certain embodimentsexamples, the glycosylation is modulated to achieve an increased G0-F (afucosylated G0), while decreasing G0 (fucosylated G0) or a decreased G0-F (afucosylated G0), while increasing G0 (fucosylated G0). In certain embodimentsexamples, glycosylation is modulated to achieve an increased or decreased afucosylation (e.g., G0-F) without impacting agalactosylation (e.g., G0). In certain embodimentsexamples, glycosylation
30 is modulated to achieve an increased or decreased agalactosylation (e.g., G0) without impacting afucosylation (e.g., G0-F)..

In certain embodimentsexamples, the subject matter disclosed herein is directed to a cell

culture composition comprising, a cell culture media and/or cell culture comprising manganese, galactose, and/or fucose supplementation (or no supplementation), high or low pCO₂, an extended or shortened media hold time, an extended or shortened culture duration, a higher or lower cultivation temperature, a higher or lower osmolality, and/or an increased or decreased Na⁺ concentration, and/or any combinations thereof; and a host cell engineered to express a glycoprotein of interest. In certain embodimentsexamples, the cell culture composition further comprises the glycoprotein of interest. In certain embodimentsexamples, the glycoprotein of interest is an antibody. In certain embodimentsexamples, the antibody is a chimeric antibody, a humanized antibody, or a human antibody. In certain embodimentsexamples, the antibody is ocrelizumab. In certain embodimentsexamples, the host cell is a mammalian cell, e.g., a CHO cell.

In certain embodimentsexamples, the subject matter disclosed herein is directed to a composition comprising a glycoprotein of interest, wherein the preparation comprises a cell culture media and/or cell cultures comprising manganese, galactose, and/or fucose supplementation (or no supplementation), high or low pCO₂, an extended or shortened media hold time, an extended or shortened culture duration, a higher or lower cultivation temperature, a higher or lower osmolality, and/or an increased or decreased Na⁺ concentration, and/or any combinations thereof; a host cell engineered to express a glycoprotein of interest; and the glycoprotein of interest. In certain embodimentsexamples, the glycoprotein of interest is an antibody. In certain embodimentsexamples, the antibody is a chimeric antibody, a humanized antibody, or a human antibody. In certain embodimentsexamples, the antibody is ocrelizumab. In certain embodimentsexamples, the host cell is a mammalian cell, e.g., a CHO cell.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts variation in Mn levels on day 0 of production cultures correlate with variation in agalactosylation, %G0 (fucosylated G0, bottom) and afucosylation, normalized %G0-F (afucosylated, top) antibody species.

Figures 2A and 2B depict effects of Day 0 manganese concentration on galactosylation and fucosylation of mAb product in Ocrelizumab cell culture process. Plots of G0 against Day 0 Mn concentration (nM) are depicted in Figure 2A. Plots of normalized G0-F against Day 0 Mn concentration (nM) are depicted in Figure 2B.

What is claimed is:

1. A method for modulating the glycosylation pattern of a glycoprotein of interest in a cell culture, comprising:

modulating ~~the following parameters, either alone or in any combination,~~ in a cell culture medium, and/or, in a cell culture environment:

- a. a Mn concentration from ~~about~~ 1 nM to ~~about~~ 20000 nM in a high partial pressure CO₂ (pCO₂) condition; and
- b. a Mn concentration from ~~about~~ 1 nM to ~~about~~ 30000 nM in a low pCO₂ condition;
- ~~c. a pCO₂ from about 10 mmHg to about 250 mmHg;~~
- ~~d. a pre-inoculation cell culture media hold duration from about 0 hrs to about 120 hrs at a temperature of about 25°C to 39°C;~~
- ~~e. a cell culture duration from about 0 days to about 150 days;~~
- ~~f. a Na⁺ concentration from about 0 mM to about 300 mM;~~
- ~~g. an osmolality from about 250 mOsm/kg to about 550 mOsm/kg;~~
- ~~h. a galactose concentration from about 0 mM to about 60 mM;~~
- ~~i. a fucose concentration from about 0 mM to about 60 mM; and~~
- ~~j. a cultivation temperature from about 29°C to about 39°C.~~

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 glycoprotein of interest is a recombinant protein, optionally wherein 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).

4. The method of claim 3, 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.

5. The method of claim 3, wherein the antibody or antibody fragment exhibits:

- i) a % GO-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 % GO-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,

ii) a % GO (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%.

6. The method of claim 3, wherein the glycosylation is modulated to achieve:

- a. an increased afucosylation (e.g., GO-F (afucosylated GO)), while decreasing agalactosylation (e.g., GO (fucosylated, agalactosylated GO)); or,
- b. a decreased afucosylation (e.g., GO-F), while increasing agalactosylation (e.g., GO); or,
- c. an increased or decreased afucosylation (e.g., GO-F) without impacting agalactosylation (e.g., GO); or,
- d. an increased or decreased agalactosylation (e.g., GO) without impacting afucosylation (e.g., GO-F).

7. 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 ~~about~~ 6.1 to ~~about~~ 7.3; or ~~about~~ 6.3 to ~~about~~ 7.3 prior to High Temperature Short Time (HTST) 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).

8. 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 ~~one or more of:~~ Mn, ~~fucose, galactose and/or Na⁺,~~ and wherein the supplementation is based on a pre-defined schedule or criteria, optionally wherein the ~~one or more of the~~ Mn, ~~fucose, galactose and Na⁺~~ 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.

9. The method of claim 1, wherein the cell culture medium consists essentially of ~~one or more of:~~ i) Mn; ii) ~~fucose;~~ iii) ~~galactose;~~ and/or iv) ~~Na⁺.~~

10. The method of claim 1, wherein modulation of the glycosylation 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.

~~11. The method of claim 1, wherein modulation of the glycosylation pattern of the glycoprotein of interest comprises:~~

~~(i) modulating the duration of the cell culture, wherein the duration of the cell culture is about 0 days to about 150 days; about 0 days to about 15 days; about 0 days to about 12 days; 0 days to about 7 days; or about 0 days to about 5 days; or~~

~~(ii) modulating the Na⁺ concentration, wherein the Na⁺ concentration is about 0 mM to about 300 mM; is about 20 mM to about 20 mM; about 30 mM to about 150 mM; or about 40 mM to about 130 mM; or~~

~~(iii) modulating the osmolality, wherein the osmolality of is about 250 mOsm/kg to about 550 mOsm/kg; about 300 mOsm/kg to about 450 mOsm/kg; or about 325 mOsm/kg to about 425 mOsm/kg; or~~

~~(iv) modulating the galactose concentration, wherein the galactose concentration is about 0 mM to about 60 mM or about 0 mM to about 50 mM; or~~

~~(v) modulating the fucose concentration, wherein the fucose concentration is about 0 mM to about 60 mM; 0 mM to about 40 mM; about 0 mM to about 20 mM; or about 0 mM to about 10 mM; or~~

~~(vi) modulating the cell culture temperature, wherein the cell culture temperature is about 29°C to about 39°C; about 30°C to about 39°C; about 31°C to about 38°C; or about 34°C to about 38°C.~~

~~12. The method of claim 1, wherein the cell culture temperature is modulated prior the production stage of the cell culture, optionally wherein the cell culture temperature is modulated based on a pre-defined schedule or criteria.~~

~~113. The method of claim 1, wherein the cell culture comprises eukaryotic cells, optionally wherein the eukaryotic cells are CHO cells.~~

~~124. The method of claim 1, wherein the cell culture is in a bioreactor, wherein the bioreactor comprises including but not limited to: 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.~~

~~153. A method to prepare a cell culture media, a feed media, a hydrolysate, or an additive comprising one or more step(s) of modulating:~~

~~a. the Mn concentration in a high partial pressure CO₂ (pCO₂) culture from about 1 nM to about 20000 nM; and~~

~~b. the Mn concentration in a low pCO₂ culture from about 1 nM to about 30000 nM;~~

~~c. the pCO₂ from about 10 mmHg to about 250 mmHg;~~

~~d. the pre-inoculation cell culture media hold duration from about 0 hrs to about 120 hrs;~~

~~e. the cell culture duration from about 0 days to about 150 days;~~

- ~~f. the Na⁺ concentration from about 0 mM to about 300 mM;~~
- ~~g. the osmolality from about 250 mOsm/kg to about 550 mOsm/kg;~~
- ~~h. the galactose concentration from about 0 mM to about 60 mM;~~
- ~~i. the fucose concentration from about 0 mM to about 60 mM; and~~
- ~~j. the cultivation temperature from about 29°C to about 39°C;~~

wherein the cell culture media, feed media, hydrolysate, or additive modulates the glycosylation pattern of a glycoprotein of interest.

146. A cell culture composition comprising,

- a. a host cell engineered to express a glycoprotein 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 ~~about~~ 1 nM to ~~about~~ 20000 nM; and
 - ii. the Mn concentration in a low pCO₂ culture from ~~about~~ 1 nM to ~~about~~ 30000 nM;
 - ~~iii. the pCO₂ from about 10 mmHg to about 250 mmHg;~~
 - ~~iv. the pre-inoculation cell culture media hold duration from about 0 hrs to about 120 hrs;~~
 - ~~v. the cell culture duration from about 0 days to about 150 days;~~
 - ~~vi. the Na⁺ concentration from about 0 mM to about 300 mM;~~
 - ~~vii. the osmolality from about 250 mOsm/kg to about 550 mOsm/kg;~~
 - ~~viii. the galactose concentration from about 0 mM to about 60 mM;~~
 - ~~ix. the fucose concentration from about 0 mM to about 60 mM; and~~
 - ~~x. the cultivation temperature from about 29°C to about 39°C.~~

157. 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 claims 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.

~~18. A method of modulating the glycosylation of a glycoprotein of interest, the method comprising:~~

~~a. assaying cell culture media to determine if the manganese concentration of the cell culture media falls within a targeted range; and~~

~~b. culture a host cell engineered to express the glycoprotein of interest in the cell culture media falling within the targeted range;~~

~~wherein the glycosylation of glycoproteins of interest is modulated as compared to the glycosylation of glycoproteins of interest expressed by the host cell in culture media falling outside the targeted range of manganese concentrations.~~

~~19. A cell culture composition comprising,~~

~~a. a cell culture media assayed to determine if the manganese concentration of the cell culture media falls within a targeted range; and~~

~~b. a host cell engineered to express a glycoprotein of interest.~~

~~20. A composition comprising a glycoprotein of interest, wherein the preparation comprises:~~

~~a. a cell culture media assayed to determine if the manganese concentration of the cell culture media falls within a targeted range;~~

~~b. a host cell engineered to express a glycoprotein of interest; and~~

~~c. the glycoprotein of interest.~~