

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;
- 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.

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

14. The method of claim 1, wherein 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, optionally wherein the volume of the cell culture is from 1 mL to 35,000 L.

15. 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;
- 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.

16. 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;
 - 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.

17. 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.