

SUMMARY

[0007] Described for the first time are data demonstrating that the concentration of fucose and/or glucose in a cell culture medium comprising cells producing a recombinant glycosylated protein (e.g., an antibody or antibody binding protein) influences the level of TAF glycoforms of the recombinant glycosylated protein produced. Whereas larger changes in the level of TAF glycoforms of a recombinant glycosylated protein (e.g., an antibody or antibody binding protein) can be achieved by manipulating the concentration of fucose in the cell culture medium comprising cells producing the recombinant glycosylated protein (e.g., antibody or antibody binding protein), smaller changes in the TAF glycoforms level can be achieved by altering the concentration of glucose in the cell culture medium, as described herein. Also, the data demonstrate that, while glucose concentration of the cell culture medium affects levels of high mannose glycans and afucosylated glycans, the fucose concentration of the cell culture medium affects levels of afucosylated glycans but does not influence high mannose glycan levels. The discovery that each of these sugars, differing in chemical formula by only one oxygen atom, leads to differential effects on TAF glycoform levels was unexpected. Without being bound to a particular theory, maintaining cells producing the recombinant glycosylated protein (e.g., antibody or antibody binding protein) in a cell culture medium comprising fucose and/or glucose at concentrations as taught herein allows for production of a recombinant glycosylated protein (e.g., an antibody or antibody binding protein) composition having a desired or predetermined or pre-selected level of TAF glycoforms (e.g., high mannose glycans and afucosylated glycans). Accordingly, the disclosure relates to methods of producing a recombinant glycosylated protein composition (e.g., an antibody composition or antibody binding protein composition) comprising a desired or predetermined or pre-selected level of TAF glycoforms.

[0007a] In one aspect, the present disclosure refers to a method of producing an antibody composition, wherein the level of total afucosylated (TAF) glycans in the antibody composition is less than ~~about~~ 10%, the method comprising maintaining glycosylation-competent cells in a cell culture medium comprising fucose, wherein fucose is present in the culture medium at a concentration of ~~about~~ 0.17 g/L to ~~about~~ 1.0 g/L. ~~In another aspect, the present disclosure refers to a method of producing an antibody composition, wherein the level of total afucosylated (TAF) glycans in the antibody composition is less than about 10%, the method comprising maintaining glycosylation-competent cells in a cell culture medium comprising fucose, wherein fucose is present in the culture medium at a concentration of about 0.1 g/L to about 1.0 g/L, and wherein the glycosylation-competent cells are not genetically modified to alter activity of an enzyme of the de novo pathway or the salvage pathway. In yet another aspect, the present disclosure refers to a method of producing an~~

antibody composition, comprising maintaining glycosylation-competent cells in a cell culture medium comprising fucose and glucose, wherein fucose is present in the culture medium at a concentration of about 0.1 g/L to about 1.0 g/L and glucose is added to the cell culture medium according to a glucose feeding schedule that achieves an average glucose concentration of about 10 g/L or less.

[0007b] In a further aspect, the present disclosure refers to an antibody composition produced by the method as described herein. In a further aspect, the present disclosure refers to a pharmaceutical composition comprising the antibody composition as described herein and a pharmaceutically acceptable carrier, diluent or excipient. In a further aspect, the present disclosure refers to a cell culture medium comprising: a. glycosylation-competent cells comprising an exogenous nucleic acid encoding an antibody; and b. a culture medium comprising fucose at a concentration of about 0.1 g/L to about 1.0 g/L or about 0.17 g/L to about 1.0 g/L, optionally wherein the glycosylation-competent cells are not genetically modified to alter activity of an enzyme of the de novo pathway or the salvage pathway, optionally, wherein the glycosylation-competent cells are not genetically modified to knock-out a gene encoding GDP-keto-6-deoxymannose-3,5-epimerase, 4-reductase.

[0007c0007b] In a further aspect, the present disclosure refers to a method of modulating the level of TAF glycans of an antibody composition produced by glycosylation-competent cells, comprising (A) adding fucose to a cell culture medium comprising the glycosylation-competent cells to achieve a fucose concentration of ~~about~~ 0.1 g/L to ~~about~~ 1.0 g/L to decrease the level of TAF glycans; or (B) adding fucose to a cell culture medium comprising the glycosylation-competent cells to achieve a fucose concentration of 0.1 g/L to 1.0 g/L to decrease the level of TAF glycans and adding glucose to a cell culture medium comprising the glycosylation-competent cells to achieve a glucose concentration less than ~~about~~ 10 g/L to increase the level of TAF glycans; or (C) ~~both (A) and (B)~~. In a still further aspect, the present disclosure refers to a method of modulating the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells, comprising (A) adding fucose to a cell culture medium comprising the glycosylation-competent cells to achieve a fucose concentration of ~~about~~ 0.1 g/L to ~~about~~ 1.0 g/L to decrease the level of afucosylated glycans; or (B) adding fucose to a cell culture medium comprising the glycosylation-competent cells to achieve a fucose concentration of 0.1 g/L to 1.0 g/L to decrease the level of afucosylated glycans and adding glucose to a cell culture medium comprising the glycosylation-competent cells to achieve a glucose concentration less than or ~~about~~ 10 g/L to increase the level of afucosylated glycans; or (C) ~~both (A) and (B)~~. In a still further aspect, ~~the present disclosure refers to a method of modulating the level of high mannose glycans of an antibody composition produced by glycosylation-competent cells, comprising adding glucose to a cell culture medium comprising the glycosylation-competent cells to achieve a glucose concentration less than or about 10 g/L to increase the level of high mannose glycans.~~

[0007d] In a further aspect, the present disclosure refers to a method of modulating the level of afucosylated glycans of an antibody composition produced by glycosylation-

competent cells comprising reducing the pH of the cell culture medium by about 0.03 to about 1.2 to reduce the level of afucosylated glycans of an antibody composition by about 0.5% to about 2% or increasing the pH of the cell culture medium by about 0.03 to about 1.2 to increase the level of afucosylated glycans of an antibody composition by about 0.5% to about 2%, optionally, wherein the method comprises (A) reducing the pH of the cell culture medium by about 0.05 to about 1.2 to reduce the level of afucosylated glycans of the antibody composition by about 1% to about 2% or increasing the pH of the cell culture medium by about 0.05 to about 1.2 to increase the level of afucosylated glycans of the antibody composition by about 1% to about 2% or (B) reducing the pH of the cell culture medium by about 0.03 to about 0.07 to reduce the level of afucosylated glycans of the antibody composition by about 0.5% to about 1.1% or increasing the pH of the cell culture medium by about 0.03 to about 0.07 to increase the level of afucosylated glycans of the antibody composition by about 0.5% to about 1.1%. In a further aspect, the present disclosure refers to a method of reducing the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells by about 1% to about 2%, comprising reducing the pH of the cell culture medium by about 0.05 to about 1.2, optionally, wherein the method comprises (A) reducing the pH by about 0.05 to about 0.07 for a reduction in afucosylated glycans of about 1%, (B) reducing the pH by about 0.09 to about 1.2 for a reduction in afucosylated glycans of more than about 1.5%, or (C) culturing the cells at a pH between about 7.10 to about 7.20, optionally about 7.12 to about 7.19. In a further aspect, the present disclosure refers to a method of reducing the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells by about 0.5% to about 1.1%, comprising reducing the pH of the cell culture medium by about 0.03-0.07, optionally, wherein the method comprises (A) reducing the pH by about 0.03 to about 0.06 for a reduction in afucosylated glycans of about 0.8%, (B) reducing the pH by about 0.05 to about 0.07 for a reduction in afucosylated glycans of about 1%, or (C) culturing the cells at a pH higher than 7.05 and less than or about 7.15, optionally about 7.07 to about 7.13. In a further aspect, the present disclosure refers to a method of increasing the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells by about 1% to about 2%, comprising increasing the pH of the cell culture medium by about 0.05 to about 1.2, optionally, wherein the method comprises (A) increasing the pH by about 0.05 to about 0.07 for a reduction in afucosylated glycans of about 1%, (B) increasing the pH by about 0.09 to about 1.2 for a reduction in afucosylated glycans of more than about 1.5%, (C) culturing the cells at a pH between about 7.10 to about 7.20, optionally about 7.12 to about 7.19. In yet another aspect, the present disclosure refers to a method of increasing the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells by about 0.5% to about 1.1%, comprising increasing the pH of the cell

~~culture medium by about 0.03-0.07, optionally, wherein the method comprises (A) increasing the pH by about 0.03 to about 0.06 for a reduction in afucosylated glycans of about 0.8%, (B) increasing the pH by about 0.05 to about 0.07 for a reduction in afucosylated glycans of about 1%, or (C) culturing the cells at a pH higher than 7.05 and lower than or about 7.15, optionally about 7.07 to about 7.13. In yet another aspect, the present disclosure refers to a method of modulating the level of TAF glycans of an antibody composition produced by glycosylation-competent cells, comprising modulating, reducing or increasing the level of afucosylated glycans of the antibody composition in accordance with a method as described herein. In yet another aspect, the present disclosure refers to a method of producing an antibody composition, wherein the level of afucosylated glycans in the antibody composition is about 6.2% to about 8.4%, the method comprising maintaining glycosylation-competent cells in a cell culture medium at a pH higher than 7.05 and lower than 7.2, wherein: (A) the pH of the cell culture medium changes by less than 0.15 (optionally by less than 0.10) during the culture period or (B) the temperature of the cell culture medium changes by not more than 2 degrees C or (C) the method does not comprise culturing the cells in a cell culture medium comprising manganese or betaine or (D) a combination of two or three of (A), (B), and (C), optionally, wherein the pH is maintained at a pH of about 7.07 to about 7.19 during the culture period, optionally, wherein the pH is maintained at about 7.07 or higher and below 7.10, or about 7.10 or higher and below 7.15, or about 7.15 or higher up to about 7.19.~~

[0008] The disclosure provides methods of producing a recombinant glycosylated protein composition (e.g., an antibody composition or antibody binding protein composition). In exemplary examples, the method comprises maintaining glycosylation-competent cells in a cell culture medium comprising fucose and/or glucose at a specific concentration as described herein, depending on the level of TAF glycoforms desired.

[0009] In exemplary examples, the level of TAF glycoforms in the recombinant glycosylated protein composition (e.g., antibody composition or antibody binding protein composition) is less than or about 10% and, in exemplary aspects, the method comprises maintaining glycosylation-competent cells in a cell culture medium comprising fucose, wherein fucose is present in the culture medium at a concentration between about 0.17 g/L and about 1.0 g/L.

[0010] In exemplary examples, the level of TAF glycoforms in the recombinant glycosylated protein composition (e.g., antibody composition or antibody binding protein composition) is less than or about 10% and, in exemplary aspects, the method comprises maintaining glycosylation-competent cells in a cell culture medium comprising fucose, wherein fucose is present in the culture medium at a concentration between about 0.1 g/L

and about 1.0 g/L, and wherein the glycosylation-competent cells are not genetically modified to alter activity of an enzyme of the de novo pathway or the salvage pathway.

[0011] The disclosure also provides methods of producing a recombinant glycosylated protein composition (e.g., an antibody composition or antibody binding protein composition) comprising maintaining glycosylation-competent cells in a cell culture medium comprising fucose and glucose, wherein fucose is present in the culture medium at a concentration of about 0.1 g/L to about 1.0 g/L and adding glucose to the cell culture medium according to a glucose feeding schedule that achieves an average glucose concentration of about 10 g/L or less.

[0012] Recombinant glycosylated protein compositions (e.g., antibody compositions or antibody binding protein compositions) produced by the methods of the disclosure are provided herein. Additionally, related pharmaceutical compositions and cell culture media are provided. In exemplary aspects, the cell culture medium comprises comprising an exogenous nucleic acid encoding an antibody (e.g., an IgG antibody) and a culture medium comprising fucose at a concentration of about 0.1 g/L to about 1.0 g/L or about 0.17 g/L to about 1.0 g/L. In some instances, the glycosylation-competent cells are not genetically modified to alter activity of an enzyme of the de novo pathway or the salvage pathway. Optionally, the glycosylation-competent cells are not genetically modified to knock-out a gene encoding GDP-keto-6-deoxymannan-3,5-epimerase, 4-reductase. The culture medium, in some aspects, further comprises glucose at a concentration less than about 10 g/L, optionally, less than about 9 g/L or about 6 g/L or less (e.g., about 0.5 g/L to about 4 g/L). In exemplary instances, the pH of the culture medium is about 6.85 to about 7.05, e.g., about 6.90 to about 7.00. In some instances, the cell culture medium does not comprise mannose. In certain aspects, the antibody is an IgG1 antibody. In exemplary aspects, the antibody is specific for a tumor-associated antigen, such as one comprising SEQ ID NO: 3.

[0013] Methods of altering or modulating the level of TAF glycans of a recombinant glycosylated protein composition (e.g., an antibody composition or antibody binding protein composition) produced by glycosylation-competent cells in a cell culture medium are further provided herein. In exemplary aspects, the method comprises (A) adding fucose to a cell culture medium comprising the glycosylation-competent cells to achieve a fucose concentration of about 0.1 g/L to about 1.0 g/L to decrease the level of TAF glycans; (B) adding glucose to a cell culture medium comprising the glycosylation-competent cells to achieve a glucose concentration less than about 10 g/L to increase the level of TAF; or (C) a combination of both (A) and (B).

[0014] Also provided are methods of modulating the level of afucosylated glycans of a recombinant glycosylated protein composition (e.g., an antibody composition or antibody binding protein composition) produced by glycosylation-competent cells. In exemplary examples, the method comprises (A) adding fucose to a cell culture medium comprising the glycosylation-competent cells to achieve a fucose concentration of about 0.1 g/L to about 1.0 g/L, to decrease the level of afucosylated glycans; (B) adding glucose to a cell culture medium comprising the glycosylation-competent cells to achieve a glucose concentration less than or about 10 g/L to increase the level of afucosylated glycans; or (C) a combination of both (A) and (B).

[0015] The present disclosure further provides a method of modulating the level of high mannose (HM) glycans of a recombinant glycosylated protein composition (e.g., an antibody composition or antibody binding protein composition) produced by glycosylation-competent cells. In exemplary examples, the method comprises adding glucose to a cell culture medium comprising the glycosylation-competent cells to achieve a glucose concentration less than about 10 g/L to increase the level of HM glycans.

[0016] Also provided by the present disclosure are methods of modulating the level of afucosylated glycans of a recombinant glycosylated protein composition (e.g., an antibody composition or antibody binding protein composition) produced by glycosylation-competent cells comprising reducing the pH of the cell culture medium by about 0.03 to about 1.2 to reduce the level of afucosylated glycans of the composition by about 0.5% to about 2% or increasing the pH of the cell culture medium by about 0.03 to about 1.2 to increase the level of afucosylated glycans of the composition by about 0.5% to about 2%.

[0017] Methods of reducing the level of afucosylated glycans of a recombinant glycosylated protein composition (e.g., an antibody composition or antibody binding protein composition) produced by glycosylation-competent cells by about 1% to about 2%, comprising reducing the pH of the cell culture medium by about 0.05 to about 1.2 are provided by the present disclosure.

[0018] Additionally provided are methods of reducing the level of afucosylated glycans of a recombinant glycosylated protein composition (e.g., an antibody composition or antibody binding protein composition) produced by glycosylation-competent cells by about 0.5% to about 1.1%, comprising reducing the pH of the cell culture medium by about 0.03-0.07.

[0019] The present disclosure further provides methods of increasing the level of afucosylated glycans of a recombinant glycosylated protein composition (e.g., an antibody composition or antibody binding protein composition) produced by glycosylation-competent

cells by about 1% to about 2%, comprising increasing the pH of the cell culture medium by about 0.05 to about 1.2.

[0020] Also provided are methods of increasing the level of afucosylated glycans of a recombinant glycosylated protein composition (e.g., an antibody composition or antibody binding protein composition) produced by glycosylation-competent cells by about 0.5% to about 1.1%, comprising increasing the pH of the cell culture medium by about 0.03-0.07.

[0021] The present disclosure further provides methods of modulating the level of TAF glycans of a recombinant glycosylated protein composition (e.g., an antibody composition or antibody binding protein composition) produced by glycosylation-competent cells, comprising modulating, reducing or increasing, the level of afucosylated glycans of the composition in accordance with a presently disclosed method of modulating, reducing or increasing, the level of afucosylated glycans.

[0022] The present disclosure provides a method of producing a recombinant glycosylated protein composition (e.g., an antibody composition or antibody binding protein composition), wherein the level of afucosylated glycans in the composition is about 6.2% to about 8.4%, the method comprising maintaining glycosylation-competent cells in a cell culture medium at a pH higher than 7.05 and lower than 7.2, wherein: (A) the pH of the cell culture medium changes by less than 0.15 (optionally by less than 0.10) during the culture period or (B) the temperature of the cell culture medium changes by not more than 2 degrees C or the method does not comprise culturing the cells in a cell culture medium comprising manganese or betaine or (D) a combination of two or three of (A), (B), and (C).

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] Figure 1A is an illustration of the three types of N-glycans (oligomannose, complex and hybrid) and commonly used symbols for such saccharides.

[0024] Figure 1B is an illustration of exemplary glycan structures.

[0025] Figure 2 is a diagram of the salvage pathway and the de novo pathway of fucose metabolism. In the salvage pathway, free L -fucose is converted to GDP-fucose, while in the de novo pathway, GDP-fucose is synthesized via three reactions catalyzed by GMD and FX. GDP-fucose is then transported from the cytosol to the Golgi lumen by GDP-Fuc Transferase and transferred to acceptor oligosaccharides and proteins. The other reaction product, GDP, is converted by a luminal nucleotide diphosphatase to guanosine 5 -

WHAT IS CLAIMED IS:

1. A method of producing an antibody composition, wherein the level of total afucosylated (TAF) glycans in the antibody composition is less than ~~about~~ 10%, the method comprising maintaining glycosylation-competent cells in a cell culture medium comprising fucose, wherein fucose is present in the culture medium at a concentration of ~~about~~ 0.17 g/L to ~~about~~ 1.0 g/L.
- ~~2. A method of producing an antibody composition, wherein the level of total afucosylated (TAF) glycans in the antibody composition is less than about 10%, the method comprising maintaining glycosylation-competent cells in a cell culture medium comprising fucose, wherein fucose is present in the culture medium at a concentration of about 0.1 g/L to about 1.0 g/L, and wherein the glycosylation-competent cells are not genetically modified to alter activity of an enzyme of the de novo pathway or the salvage pathway.~~
- ~~3. A method of producing an antibody composition, comprising maintaining glycosylation-competent cells in a cell culture medium comprising fucose and glucose, wherein fucose is present in the culture medium at a concentration of about 0.1 g/L to about 1.0 g/L and glucose is added to the cell culture medium according to a glucose feeding schedule that achieves an average glucose concentration of about 10 g/L or less.~~
- 4.2. _____ The method of ~~any one of claims 1 to 3~~claim 1, wherein fucose is present in the culture medium at a concentration less than ~~about~~ 0.75 g/L.
- 5.3. _____ The method of claim ~~42~~2, wherein fucose is present in the culture medium at a concentration less than ~~about~~ 0.6 g/L, optionally, ~~about~~ 0.2 g/L to ~~about~~ 0.5 g/L.
- 6.4. _____ The method of any one of claims 1 to ~~53~~5, wherein the fucose is present in the culture medium the entire duration the glycosylation-competent cells are maintained in cell culture.
- 7.5. _____ The method of any one of claims 1 to ~~54~~5, comprising maintaining the glycosylation-competent cells in a first cell culture medium for an initial time period and subsequently maintaining the glycosylation-competent cells in a second cell culture medium, wherein the first cell culture medium does not comprise fucose at a concentration of ~~about~~ 0.1 g/L to ~~about~~ 1.0 g/L and the second cell culture medium comprises fucose at a concentration of ~~about~~ 0.1 g/L to ~~about~~ 1.0 g/L.
- 8.6. _____ The method of claim ~~75~~5, wherein the initial time period is ~~about~~ 24 to ~~about~~ 72 hours or ~~about~~ or greater than ~~about~~ 72 hours but less than or ~~about~~ 156 hours.

- ~~9.7.~~ The method of any one of claims ~~7-5~~ or ~~86~~, wherein fucose is added to the first culture medium on the 6th day post-cell culture inoculation to obtain the second cell culture medium.
8. The method of any one of claims 1 to 97, wherein the concentration of fucose fluctuates by ~~about~~ 0.2 g/L or less during the time the glycosylation-competent cells are maintained in the cell culture medium comprising fucose, ~~optionally,~~
- ~~10.9.~~ The method of claim 8, wherein the concentration of fucose fluctuates by ~~about~~ 0.1 g/L or less during the time the glycosylation-competent cells are maintained in the cell culture medium comprising fucose.
- ~~11.10.~~ The method of any one of claims 1 to ~~109~~, wherein the cell culture medium comprises an initial glucose concentration for an initial time period.
11. The method of claim ~~11.10~~, wherein the initial glucose concentration is ~~about~~ 1 g/L to ~~about~~ 15 g/L, ~~optionally, about 12 g/L ± 1 g/L.~~
12. The method of claim 11, wherein the initial glucose concentration is 12 g/L ± 1 g/L.
- ~~13. The method of any one of claims 1, 2, and 4 to 12, further comprising adding glucose to the cell culture medium according to a glucose feeding schedule.~~
- ~~14. The method of claim 13, wherein the glucose feeding schedule is initiated at about 4 to about 6 days post-cell culture inoculation, optionally, at about 6 days post cell culture inoculation.~~
- ~~15. The method of any one of claims 3, 13, and 14, wherein the glucose feeding schedule achieves an average glucose concentration of about 9 g/L or less, optionally, about 6 g/L or less, in the cell culture medium.~~
- ~~16. The method of claim 15, wherein the glucose feeding schedule achieves an average glucose concentration of about 0.5 g/L to about 4 g/L in the cell culture medium.~~
- ~~17. The method of any one of claims 13 to 16, wherein the glucose feeding schedule achieves an average glucose concentration based on the concentration of fucose in the cell culture medium, optionally, wherein the average glucose concentration is calculated based on Formula I:~~

$$T = 3.354 - 1.388F + 0.111G + [F - 0.4375] \times [1.9527(F - 0.4375)]$$

(Formula I)

~~wherein T is the targeted % total afucosylated (TAF) glycans in the antibody composition and is about 2.5% to about 6%, about 2.75% to about 5.5%, or about 3%~~

- to about 5%, F is the concentration (g/L) of fucose in the medium, and G is the average glucose concentration (g/L) in the medium.
- ~~18. The method of claim 17, wherein (i) the concentration of fucose is about 0.2 ± 0.1 g/L and the average glucose concentration is about 2 to about 4 g/L; (ii) the concentration of fucose is about 0.5 ± 0.1 g/L and the average glucose concentration is about 3 to about 6 g/L; or (iii) the concentration of fucose is about 0.75 ± 0.1 g/L and the average glucose concentration is about 4.5 to about 9 g/L.~~
- ~~19. The method of any one of claims 1 to 18, wherein the pH of the cell culture medium is about 6.85 to about 7.05, optionally, about 6.90 to about 7.00.~~
- ~~20. The method of any one of claims 1 and 3 to 19, wherein the glycosylation-competent cells are not genetically modified to alter activity of an enzyme of the de novo pathway or the salvage pathway.~~
- ~~21. The method of claims 2 and 20, wherein the glycosylation-competent cells are not genetically modified to knock-out a gene encoding GDP-keto-6-deoxymannose-3,5-epimerase, 4-reductase.~~
- ~~22. The method of any one of claims 3 to 21, wherein the level of total afucosylated (TAF) glycans in the antibody composition is less than about 10%.~~
13. The method of any one of claims 1 to ~~22~~12, wherein the level of TAF glycans is ~~about 2% to about 6%, optionally, about 2% to about 5% or about 2% to about 4%.~~
- ~~23-14.~~ The method of claim 13, wherein the level of TAF glycans is 2% to 5% or 2% to 4%.
15. The method of any one ~~of~~ claims 1 to ~~23~~14, wherein the level of high mannose glycans and/or the level of afucosylated glycans in the antibody composition is less than ~~about 3.5%, optionally,~~
16. The method of claim 15, wherein the antibody composition has a level of high mannose glycans ~~in the antibody composition is of~~ ~~about 0.7% to about 3.0%.~~
- ~~24-17.~~ The method of any one of claims 1 to 16, wherein and/or the antibody composition has a level of afucosylated glycans ~~in the antibody composition is of~~ ~~about 0.8% to about 2.8%.~~
- ~~25-18.~~ The method of any one of claims 1 to ~~24~~17, wherein the glycosylation-competent cells produce IgG antibodies, optionally, IgG1 antibodies.
19. The method of claim ~~25~~18, wherein the IgG1 antibodies are specific for a tumor-associated antigen.

~~26-20.~~ The method of claim 19, ~~optionally,~~ wherein the tumor-associated antigen comprises SEQ ID NO. 3.

~~27-21.~~ The method of any one of claims 1 to 2620, wherein the culture medium does not comprise mannose.

~~28. An antibody composition produced by the method of any one of claims 1 to 27.~~

~~29. A pharmaceutical composition comprising the antibody composition of claim 28 and a pharmaceutically acceptable carrier, diluent or excipient.~~

~~30. A cell culture medium comprising:~~

- ~~a. glycosylation-competent cells comprising an exogenous nucleic acid encoding an antibody; and~~
- ~~b. a culture medium comprising fucose at a concentration of about 0.1 g/L to about 1.0 g/L or about 0.17 g/L to about 1.0 g/L,~~

~~optionally wherein the glycosylation-competent cells are not genetically modified to alter activity of an enzyme of the de novo pathway or the salvage pathway, optionally, wherein the glycosylation-competent cells are not genetically modified to knock-out a gene encoding GDP-keto-6-deoxymannose-3,5-epimerase, 4-reductase.~~

~~31. The cell culture medium of claim 30, wherein the culture medium further comprises glucose at a concentration less than about 10 g/L, optionally less than about 9 g/L.~~

~~32. The cell culture medium of claim 31, wherein the concentration of glucose is about 6 g/L or less optionally about 0.5 g/L to about 4 g/L.~~

~~33. The cell culture medium of any one of claims 30 to 32, wherein the pH of the culture medium is about 6.85 to about 7.05, optionally, about 6.90 to about 7.00.~~

~~34. The cell culture medium of any one of claims 30 to 33, wherein the cell culture medium does not comprise mannose.~~

~~35. The cell culture medium of any one of claims 30 to 34, wherein the antibody is an IgG antibody, optionally, an IgG1 antibody.~~

~~36. The cell culture medium of claim 35, wherein the IgG1 antibody is specific for a tumor-associated antigen, optionally, comprising SEQ ID NO: 3.~~

~~37-22.~~ A method of modulating the level of TAF glycans of an antibody composition produced by glycosylation-competent cells, comprising (A) adding fucose to a cell culture medium comprising the glycosylation-competent cells to achieve a fucose concentration of ~~about~~ 0.1 g/L to ~~about~~ 1.0 g/L to decrease the level of TAF glycans; or (B) adding fucose to a cell culture medium comprising the glycosylation-competent cells to achieve a fucose concentration of 0.1 g/L to 1.0 g/L to decrease the level of

TAF glycans and adding glucose to a cell culture medium comprising the glycosylation-competent cells to achieve a glucose concentration less than ~~about~~ 10 g/L to increase the level of TAF glycans; ~~or (C) both (A) and (B).~~

~~38-23.~~ A method of modulating the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells, comprising (A) adding fucose to a cell culture medium comprising the glycosylation-competent cells to achieve a fucose concentration of ~~about~~ 0.1 g/L to ~~about~~ 1.0 g/L to decrease the level of afucosylated glycans; or (B) adding fucose to a cell culture medium comprising the glycosylation-competent cells to achieve a fucose concentration of 0.1 g/L to 1.0 g/L to decrease the level of afucosylated glycans and adding glucose to a cell culture medium comprising the glycosylation-competent cells to achieve a glucose concentration less than or ~~about~~ 10 g/L to increase the level of afucosylated glycans; ~~or (C) both (A) and (B).~~

~~39. A method of modulating the level of high mannose glycans of an antibody composition produced by glycosylation-competent cells, comprising adding glucose to a cell culture medium comprising the glycosylation-competent cells to achieve a glucose concentration less than or about 10 g/L to increase the level of high mannose glycans.~~

~~40. The method of any one of claims 37-39, wherein the glycosylation-competent cells are not genetically modified to alter activity of an enzyme of the de novo pathway or the salvage pathway, optionally, wherein the glycosylation-competent cells are not genetically modified to knock-out a gene encoding GDP-keto-6-deoxymannose-3,5-epimerase, 4-reductase.~~

~~41. The method of any one of claims 37-40, wherein the level of TAF glycans in the antibody composition is less than or about 10%, optionally, wherein the level of TAF glycans is about 2% to about 6%, about 2% to about 5%, or about 2% to about 4%.~~

~~42. The method of any one of claims 37-41, wherein the level of high mannose glycans in the antibody composition is less than or about 3.5%, optionally, about 0.7% to about 3.0%.~~

~~43. The method of any one of claims 37-42, wherein the level of afucosylated glycans in the antibody composition is less than or about 3.5%, optionally, wherein the level of afucosylated glycans in the antibody composition is about 0.8% to about 2.8%.~~

~~44. The method of any one of claims 37-43, wherein the fucose concentration is about 0.17 g/L to about 1.0 g/L, optionally, wherein the fucose concentration is about 0.2 g/L to about 0.5 g/L.~~

45. ~~The method of any one of claims 37 to 44, further comprising adding glucose to the cell culture medium according to a glucose feeding schedule that achieves an average glucose concentration of about 10 g/L, optionally less, optionally, wherein the average glucose concentration is less than about 6.0 g/L, optionally, less than about 4.0 g/L.~~
46. ~~The method of claim 45, wherein the average glucose concentration is based on the fucose concentration of the cell culture medium, optionally, wherein the average glucose concentration is calculated based on Formula I:~~

$$T = 3.354 - 1.388F + 0.111G + [F - 0.4375] \times [1.9527(F - 0.4375)]$$

Formula I

wherein T is the targeted % total afucosylated (TAF) glycans in an antibody composition and is about 3% to about 5%, F is the concentration (g/L) of fucose in the medium, and G is the average glucose concentration (g/L).

47. ~~A method of modulating the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells comprising reducing the pH of the cell culture medium by about 0.03 to about 1.2 to reduce the level of afucosylated glycans of an antibody composition by about 0.5% to about 2% or increasing the pH of the cell culture medium by about 0.03 to about 1.2 to increase the level of afucosylated glycans of an antibody composition by about 0.5% to about 2%, optionally, wherein the method comprises (A) reducing the pH of the cell culture medium by about 0.05 to about 1.2 to reduce the level of afucosylated glycans of the antibody composition by about 1% to about 2% or increasing the pH of the cell culture medium by about 0.05 to about 1.2 to increase the level of afucosylated glycans of the antibody composition by about 1% to about 2% or (B) reducing the pH of the cell culture medium by about 0.03 to about 0.07 to reduce the level of afucosylated glycans of the antibody composition by about 0.5% to about 1.1% or increasing the pH of the cell culture medium by about 0.03 to about 0.07 to increase the level of afucosylated glycans of the antibody composition by about 0.5% to about 1.1%.~~
48. ~~A method of reducing the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells by about 1% to about 2%, comprising reducing the pH of the cell culture medium by about 0.05 to about 1.2, optionally, wherein the method comprises (A) reducing the pH by about 0.05 to about 0.07 for a reduction in afucosylated glycans of about 1%, (B) reducing the pH by about 0.09 to about 1.2 for a reduction in afucosylated glycans of more than about 1.5%, or (C)~~

~~culturing the cells at a pH between about 7.10 to about 7.20, optionally about 7.12 to about 7.19.~~

- ~~49. A method of reducing the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells by about 0.5% to about 1.1%, comprising reducing the pH of the cell culture medium by about 0.03-0.07, optionally, wherein the method comprises (A) reducing the pH by about 0.03 to about 0.06 for a reduction in afucosylated glycans of about 0.8%, (B) reducing the pH by about 0.05 to about 0.07 for a reduction in afucosylated glycans of about 1%, or (C) culturing the cells at a pH higher than 7.05 and less than or about 7.15, optionally about 7.07 to about 7.13.~~
- ~~50. A method of increasing the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells by about 1% to about 2%, comprising increasing the pH of the cell culture medium by about 0.05 to about 1.2, optionally, wherein the method comprises (A) increasing the pH by about 0.05 to about 0.07 for a reduction in afucosylated glycans of about 1%, (B) increasing the pH by about 0.09 to about 1.2 for a reduction in afucosylated glycans of more than about 1.5%, (C) culturing the cells at a pH between about 7.10 to about 7.20, optionally about 7.12 to about 7.19.~~
- ~~51. A method of increasing the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells by about 0.5% to about 1.1%, comprising increasing the pH of the cell culture medium by about 0.03-0.07, optionally, wherein the method comprises (A) increasing the pH by about 0.03 to about 0.06 for a reduction in afucosylated glycans of about 0.8%, (B) increasing the pH by about 0.05 to about 0.07 for a reduction in afucosylated glycans of about 1%, or (C) culturing the cells at a pH higher than 7.05 and lower than or about 7.15, optionally about 7.07 to about 7.13.~~

52.24. The method of any one of the preceding claims, wherein (A) the pH of the cell culture medium throughout the culture is greater than 7.0, optionally, higher than 7.05 and lower than 7.2, (B) the level of afucosylated glycans in the antibody composition is less than ~~about~~ 10%, optionally, wherein the level of afucosylated glycans in the antibody composition is ~~about~~ 6.2% to ~~about~~ 8.4%, (C) the temperature changes by less than 2 degrees C during the culture period, optionally, wherein the temperature changes by not more than 1.5 or 1.0 degrees C during the culture period, (D) the cell culture medium does not comprise any detectable amounts of manganese or betaine, (E) the cell culture medium comprises ~~about~~ 0.10 g/L to ~~about~~ 1.0 g/L fucose,

optionally, ~~about~~ 0.17 to ~~about~~ 1.0 g/L fucose, and optionally wherein fucose is present in the culture medium at a concentration less than ~~about~~ 0.75 g/L, less than ~~about~~ 0.6 g/L, or ~~about~~ 0.2 g/L to ~~about~~ 0.5 g/L, (F) the glycosylation-competent cells are not genetically modified to alter activity of an enzyme of the de novo pathway or the salvage pathway, (G) glucose is added to the cell culture medium according to a glucose feeding schedule that achieves an average glucose concentration of ~~about~~ 10 g/L or less, or (H) any combination thereof.

~~53-25.~~ A method of modulating the level of TAF glycans of an antibody composition produced by glycosylation-competent cells, comprising modulating, reducing or increasing the level of afucosylated glycans of the antibody composition in accordance with a method of ~~any one of claims 47-52~~claim 24.

~~54. A method of producing an antibody composition, wherein the level of afucosylated glycans in the antibody composition is about 6.2% to about 8.4%, the method comprising maintaining glycosylation-competent cells in a cell culture medium at a pH higher than 7.05 and lower than 7.2,~~

~~wherein:~~

~~(A) the pH of the cell culture medium changes by less than 0.15~~

~~(optionally by less than 0.10) during the culture period or~~

~~(B) the temperature of the cell culture medium changes by not more than 2 degrees C or~~

~~(C) the method does not comprise culturing the cells in a cell culture medium comprising manganese or betaine or~~

~~(D) a combination of two or three of (A), (B), and (C),~~

~~optionally, wherein the pH is maintained at a pH of about 7.07 to about 7.19 during the culture period, optionally, wherein the pH is maintained at about 7.07 or higher and below 7.10, or about 7.10 or higher and below 7.15, or about 7.15 or higher up to about 7.19.~~

26. The method of claim 22 or 23, wherein the fucose is present in the culture medium the entire duration the glycosylation-competent cells are maintained in cell culture.

27. The method of any one of claims 22-24 and 26, comprising maintaining the glycosylation-competent cells in a first cell culture medium for an initial time period and subsequently maintaining the glycosylation-competent cells in a second cell culture medium, wherein the first cell culture medium does not comprise fucose at a

- concentration of 0.1 g/L to 1.0 g/L and the second cell culture medium comprises fucose at a concentration of 0.1 g/L to 1.0 g/L.
28. The method of claim 27, wherein the initial time period is 24 to 72 hours or greater than 72 hours but less than 156 hours.
29. The method of any one of claims 27 or 28, wherein fucose is added to the first culture medium on the 6th day post-cell culture inoculation to obtain the second cell culture medium.
30. The method of any one of claims 22-24 and 26-29, wherein the concentration of fucose fluctuates by 0.2 g/L or less during the time the glycosylation-competent cells are maintained in the cell culture medium comprising fucose.
31. The method of claim 30, wherein the concentration of fucose fluctuates by 0.1 g/L or less during the time the glycosylation-competent cells are maintained in the cell culture medium comprising fucose.
32. The method of any one of claims 22-24 and 26-31, wherein the cell culture medium comprises an initial glucose concentration for an initial time period.
33. The method of claim 32, wherein the initial glucose concentration is 1 g/L to 15 g/L.
34. The method of claim 33, wherein the initial glucose concentration is 12 g/L $\pm$ 1 g/L.
35. The method of any one of claims 22-24 and 26-34, wherein the level of TAF glycans is 2% to 6%.
36. The method of claim 35, wherein the level of TAF glycans is 2% to 5% or 2% to 4%.
37. The method of any one claims 22-24 and 26-36, wherein the level of high mannose glycans and/or the level of afucosylated glycans in the antibody composition is less than 3.5%.
38. The method of claim 37, wherein the level of high mannose glycans in the antibody composition is 0.7% to 3.0%.
39. The method of any one of claims 22-24 and 26-38, wherein the level of afucosylated glycans in the antibody composition is 0.8% to 2.8%.
40. The method of any one of claims 22-24 and 26-39, wherein the glycosylation-competent cells produce IgG antibodies, optionally, IgG1 antibodies.
41. The method of claim 40, wherein the IgG1 antibodies are specific for a tumor-associated antigen.
42. The method of claim 41, wherein the tumor-associated antigen comprises SEQ ID NO. 3.

~~55-43.~~ The method of any one of claims 22-24 and 26-42, wherein the culture medium does not comprise mannose.