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[Continued on next page]

(54) Title: METHODS OF CELL CULTURE

(57) Abstract: Polypeptide preparations having target levels of glycans, and methods of producing such polypeptide preparations using ammonium and/or lysine, are described.

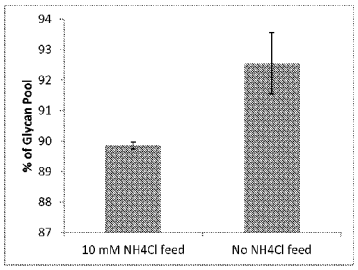

FIG. 2A

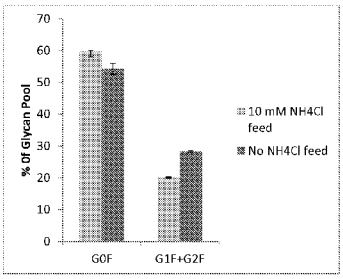

FIG. 2B

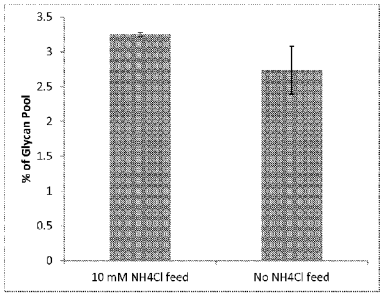

FIG. 2C

WO 2014/159488 A1

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METHODS OF CELL CULTURE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Application No. 13/829,249, filed March 14, 2013, the contents of which are hereby incorporated herein in their entirety.

FIELD OF THE INVENTION

[0002] The invention relates generally to cell culture methods.

BACKGROUND

[0003] Therapeutic polypeptides are an important class of therapeutic biotechnology products, and therapeutic antibodies (including murine, chimeric, humanized and human antibodies and fragments thereof) account for the majority of therapeutic biologic products.

SUMMARY

[0004] In one aspect, the invention features a method of producing a recombinant protein preparation having a target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans, the method comprising: (a) providing a cell genetically engineered to express a recombinant protein; (b) culturing the cell in a culture medium comprising (e.g., supplemented with) ammonium under conditions in which the cell expresses the recombinant protein; and (c) harvesting (e.g., purifying or isolating from the cell and/or culture medium) a preparation of the recombinant protein produced by the cell, wherein the preparation has the target value of the one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans. In some embodiments, the culture medium comprises ammonium for a time and in an amount effective to modify (e.g., increase or decrease) one or more of the high mannose glycans, galactosylated glycans, and fucosylated glycans of the recombinant protein.

[0005] In some embodiments, the culture medium comprises at least about 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or more, ammonium. In some embodiments, the culture medium comprises about 1 mM to about 50 mM ammonium, e.g., about 1 mM to about 10 mM ammonium, about 10 mM to about 20 mM ammonium, about 11 mM to about 19 mM ammonium, about 12 mM to about 18 mM ammonium, about 13 mM to about 17 mM ammonium, about 14 mM to about 16 mM ammonium, about 20 mM to about 30 mM ammonium, about 30 mM to about 40 mM ammonium, about 40 mM to about 50 mM ammonium, about 1 mM to about 25 mM ammonium, about 25 mM to about 50 mM ammonium, about 1 mM to about 20 mM ammonium, about 1 mM to about 30 mM ammonium, about 1 mM to about 40 mM ammonium, about 10 mM to about 50 mM ammonium, about 20 mM to about 50 mM ammonium, or about 30 mM to about 50 mM ammonium.

[0006] In some embodiments, the target value is a level of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans in a reference therapeutic product. In some embodiments, the target value is a level of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans in a reference therapeutic antibody product. In some embodiments, the target value is a predetermined pharmaceutical product specification or a quality control criterion for a pharmaceutical preparation, e.g., a Certificate of Analysis (CofA), a Certificate of Testing (CofT), or a Master Batch Record. In some embodiments, the product specification is a product description in an FDA label, a Physician's Insert, a USP monograph, or an EP monograph.

[0007] In some embodiments, the reference therapeutic product is selected from the group consisting of: abatacept, abciximab, adalimumab, aflibercept, alefacept, alemtuzumab, basiliximab, bevacizumab, belatacept, certolizumab, cetuximab, daclizumab, eculizumab, efalizumab, entanercept, gemtuzumab, ibritumomab, infliximab, muromonab-CD3, natalizumab, omalizumab, palivizumab; panitumumab, ranibizumab, rilonacept, rituximab, tositumomab, and trastuzumab.

[0008] In some embodiments, the target value is one or more of: (a) at least about 0.1% to about 20% high mannose glycans, e.g., at least about 0.1%, about 0.5%, about 1%, about 2%,

about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, or more, high mannose glycans; (b) at least about 1% to about 95% galactosylated glyans, e.g., at least about 1%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 95%, galactosylated glycans; and (c) at least about 70% to 100% fucosylated glycans, e.g., at least about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% fucosylated glycans. High mannose glycans can be, e.g., HM3, HM4, HM5, HM6, HM7, HM8, HM9, or combinations thereof.

[0009] In some embodiments, the target value of the one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans is higher than a corresponding level in a preparation produced by culturing the cell in the medium not supplemented with ammonium, e.g., not comprising an elevated level of ammonium. In some embodiments, the target value is higher than the corresponding level by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, or more, of the corresponding level.

[0010] In some embodiments, the method further comprises evaluating the level of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans in the recombinant protein preparation. In some embodiments, the method further comprises recording the level in a print or computer-readable medium, e.g., in a test report, Material Safety Data Sheet (MSDS), batch record, Certificate of Testing (CofT) or Certificate of Analysis (CofA).

[0011] In another aspect, the invention features a method of producing a recombinant protein preparation, the method comprising: (a) providing a target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans; (b) providing a cell genetically engineered to express a recombinant protein; (c) culturing the cell in a culture medium comprising (e.g., supplemented with) ammonium under conditions in which the cell expresses the recombinant protein; (d) harvesting a preparation of the recombinant protein produced by the cell; and (e) processing (e.g., one or more of formulating, filling into a container, labeling, packaging) the preparation into a drug product if the preparation meets the target value of the one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans. In some

embodiments, the culture medium comprises ammonium (e.g., an elevated level of ammonium) for a time and in an amount effective to modify (e.g., increase or decrease) one or more of the high mannose glycans, galactosylated glycans, and fucosylated glycans of the recombinant protein.

[0012] In some embodiments, the method further comprises evaluating the level of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans in the recombinant protein preparation. In some embodiments, the method further comprises recording the level in a print or computer-readable medium, e.g., in a test report, Material Safety Data Sheet (MSDS), batch record, or Certificate of Testing (CofT) or Certificate of Analysis (CofA).

[0013] In some embodiments, the culture medium comprises at least about 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or more, ammonium. In some embodiments, the culture medium comprises about 1 mM to about 50 mM ammonium, e.g., about 1 mM to about 10 mM ammonium, about 10 mM to about 20 mM ammonium, about 11 mM to about 19 mM ammonium, about 12 mM to about 18 mM ammonium, abouat 13 mM to about 17 mM ammonium, about 14 mM to about 16 mM ammonium, about 20 mM to about 30 mM ammonium, about 30 mM to about 40 mM ammonium, about 40 mM to about 50 mM ammonium, about 1 mM to about 25 mM ammonium, about 25 mM to about 50 mM ammonium, about 1 mM to about 20 mM ammonium, about 1 mM to about 30 mM ammonium, about 1 mM to about 40 mM ammonium, about 10 mM to about 50 mM ammonium, about 20 mM to about 50 mM ammonium, or about 30 mM to about 50 mM ammonium.

[0014] In some embodiments, the target value is a level of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans in a reference therapeutic product. In some embodiments, the target value is a level of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans in a reference therapeutic antibody product. In some embodiments, the target value is a predetermined pharmaceutical product specification or a quality control criterion for a pharmaceutical preparation, e.g., a Certificate of Analysis (CofA), a Certificate of Testing (CofT), or a Master Batch Record. In some embodiments, the product

specification is a product description in an FDA label, a Physician's Insert, a USP monograph, or an EP monograph.

[0015] In some embodiments, the reference therapeutic product is selected from the group consisting of: abatacept, abciximab, adalimumab, aflibercept, alefacept, alemtuzumab, basiliximab, bevacizumab, belatacept, certolizumab, cetuximab, daclizumab, eculizumab, efalizumab, entanercept, gemtuzumab, ibritumomab, infliximab, muromonab-CD3, natalizumab, omalizumab, palivizumab; panitumumab, ranibizumab, rilonacept, rituximab, tositumomab, and trastuzumab.

[0016] In some embodiments, the target value is one or more of: (a) at least about 0.1% to about 20% high mannose glycans, e.g., at least about 0.1%, about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, or more, high mannose glycans; (b) at least about 1% to about 95% galactosylated glyans, e.g., at least about 1%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 95%, galactosylated glycans; and (c) at least about 70% to 100% fucosylated glycans, e.g., at least about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% fucosylated glycans. High mannose glycans can be, e.g., HM3, HM4, HM5, HM6, HM7, HM8, HM9, or combinations thereof.

[0017] In some embodiments, the target value of the one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans is higher than a corresponding level in a preparation produced by culturing the cell in the medium not comprising an elevated level of ammonium. In some embodiments, the target value is higher than the corresponding level by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, or more, of the corresponding level.

[0018] In another aspect, the invention features a method of increasing a level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans in a recombinant protein preparation, the method comprising: (a) providing a cell genetically engineered to express a recombinant protein; (b) culturing the cell in a culture medium comprising an elevated of ammonium (e.g., supplemented with ammonium) under conditions in which the cell expresses

the recombinant protein; and (c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has an increased level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans relative to a corresponding level in a preparation of the recombinant protein produced by culturing the cell in the medium not comprising the elevated level of ammonium. In some embodiments, the culture medium comprises an elevated level of ammonium for a time and in an amount effective to increase one or more of the high mannose glycans, afucosylated glycans, and G0F glycans of the recombinant protein. In some embodiments, the method further comprises processing, (e.g., one or more of formulating, filling into a container, labeling, packaging) the preparation into a drug product if the preparation meets a target value of one or more of high mannose glycans, afucosylated glycans, and G0F glycans.

[0019] In some embodiments, the culture medium comprises at least about 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or more, ammonium. In some embodiments, the culture medium comprises about 1 mM to about 50 mM ammonium, e.g., about 1 mM to about 10 mM ammonium, about 10 mM to about 20 mM ammonium, about 11 mM to about 19 mM ammonium, about 12 mM to about 18 mM ammonium, about 13 mM to about 17 mM ammonium, about 14 mM to about 16 mM ammonium, about 20 mM to about 30 mM ammonium, about 30 mM to about 40 mM ammonium, about 40 mM to about 50 mM ammonium, about 1 mM to about 25 mM ammonium, about 25 mM to about 50 mM ammonium, about 1 mM to about 20 mM ammonium, about 1 mM to about 30 mM ammonium, about 1 mM to about 40 mM ammonium, about 10 mM to about 50 mM ammonium, about 20 mM to about 50 mM ammonium, or about 30 mM to about 50 mM ammonium.

[0020] In some embodiments, the method further comprises measuring a level of the one or more of high mannose glycans, afucosylated glycans, and G0F glycans in the recombinant protein preparation. In some embodiments, the method further comprises recording the level in a print or computer-readable medium, e.g., in a test report, Material Safety Data Sheet (MSDS), batch record, Certificate of Testing (CofT) or Certificate of Analysis (CofA).

[0021] In some embodiments, the increased level of the one or more of high mannose glycans, afucosylated glycans, and G0F glycans is higher than the corresponding level by at least about

10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, or more, of the corresponding level. In some embodiments, the increased level is one or more of: (a) at least about 0.1% to about 20% high mannose glycans, e.g., at least about 0.1%, about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, or more, high mannose glycans; (b) at least about 70% to 100% fucosylated glycans, e.g., at least about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% fucosylated glycans; and (c) at least about 0.1% to about 90% G0F glycans, e.g., at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% G0F glycans. High mannose glycans can be, e.g., HM3, HM4, HM5, HM6, HM7, HM8, HM9, or combinations thereof.

[0022] In another aspect, the invention features a method of decreasing a level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans in a recombinant protein preparation, the method comprising: (a) providing a cell genetically engineered to express a recombinant protein; (b) culturing the cell in a culture medium comprising a reduced level of ammonium under conditions in which the cell expresses the recombinant protein; and (c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has a decreased level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans relative to a corresponding level in a preparation of the recombinant protein produced by culturing the cell in the medium not comprising the reduced level of ammonium. In some embodiments, the culture medium comprises the reduced level of ammonium for a time and in an amount effective to decrease a level of one or more of the high mannose glycans, afucosylated glycans, and G0F glycans of the recombinant protein. In some embodiments, the method further comprises processing, (e.g., one or more of formulating, filling into a container, labeling, packaging) the preparation into a drug product if the preparation meets a target value of one or more of high mannose glycans, afucosylated glycans, and G0F glycans.

[0023] In some embodiments, the medium comprises less than about 50 mM, 45 mM, 40 mM, 35 mM, 30 mM, 25 mM, 20 mM, 15 mM, 10 mM, 5 mM, 1 mM, 0.5 mM, 0.1 mM, or less, ammonium.

[0024] In some embodiments, the method further comprises measuring a level of the one or more of high mannose glycans, afucosylated glycans, and G0F glycans in the recombinant protein preparation. In some embodiments, the method further comprises recording the level in a print or computer-readable medium, e.g., in a test report, Material Safety Data Sheet (MSDS), batch record, Certificate of Testing (CofT) or Certificate of Analysis (CofA).

[0025] In some embodiments, the decreased level of the one or more of high mannose glycans, afucosylated glycans, and G0F glycans is lower than the corresponding level by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, of the corresponding level. In some embodiments, the decreased level is one or more of: (a) less than about 20% high mannose glycans; (b) less than about 90% fucosylated glycans; and (c) less than about 90% G0F glycans. High mannose glycans can be, e.g., HM3, HM4, HM5, HM6, HM7, HM8, HM9, or combinations thereof.

[0026] In another aspect, the invention features a method of decreasing a level of one or more of galactosylated glycans and fucosylated glycans in a recombinant protein preparation, the method comprising: (a) providing a cell genetically engineered to express a recombinant protein; (b) culturing the cell in a culture medium comprising an elevated level of ammonium (e.g., supplemented with ammonium) under conditions in which the cell expresses the recombinant protein; and (c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has a decreased level of one or more of galactosylated glycans and fucosylated glycans relative to a corresponding level in a preparation of the recombinant protein produced by culturing the cell in the medium not comprising the elevated level of ammonium. In some embodiments, the culture medium comprises an elevated level of ammonium for a time and in an amount effective to decrease the level of one or more of galactosylated glycans and fucosylated glycans of the recombinant protein. In some embodiments, the method further comprises processing, (e.g., one or more of formulating, filling into a container, labeling, packaging) the preparation into a drug product if the preparation meets a target value of one or more of galactosylated glycans and fucosylated glycans.

[0027] In some embodiments, the culture medium comprises at least about 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50

mM, or more, ammonium. In some embodiments, the culture medium comprises about 1 mM to about 50 mM ammonium, e.g., about 1 mM to about 10 mM ammonium, about 10 mM to about 20 mM ammonium, about 11 mM to about 19 mM ammonium, about 12 mM to about 18 mM ammonium, about 13 mM to about 17 mM ammonium, about 14 mM to about 16 mM ammonium, about 20 mM to about 30 mM ammonium, about 30 mM to about 40 mM ammonium, about 40 mM to about 50 mM ammonium, about 1 mM to about 25 mM ammonium, about 25 mM to about 50 mM ammonium, about 1 mM to about 20 mM ammonium, about 1 mM to about 30 mM ammonium, about 1 mM to about 40 mM ammonium, about 10 mM to about 50 mM ammonium, about 20 mM to about 50 mM ammonium, or about 30 mM to about 50 mM ammonium.

[0028] In some embodiments, the method further comprises measuring a level of one or more of galactosylated glycans and fucosylated glycans in the recombinant protein preparation. In some embodiments, the method further comprises recording the level in a print or computer-readable medium, e.g., in a test report, Material Safety Data Sheet (MSDS), batch record, Certificate of Testing (CofT) or Certificate of Analysis (CofA).

[0029] In some embodiments, the decreased level of the one or more of galactosylated glycans and fucosylated glycans is lower than the corresponding level by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, of the corresponding level. In some embodiments, the decreased level is less than about 100% fucosylated glycans, e.g., less than about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, or 10%, fucosylated glycans. In some embodiments, the one or more fucosylated glycans comprise one or more of G1F glycans and G2F glycans. In some embodiments, the decreased level is less than about 95% galactosylated glycans, e.g., less than about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 1%, or 0.5%, galactosylated glycans.

[0030] In another aspect, the invention features a method of increasing a level of one or more of galactosylated glycans and fucosylated glycans in a recombinant protein preparation, the method comprising: (a) providing a cell genetically engineered to express a recombinant protein; (b) culturing the cell in a culture medium comprising a reduced level of ammonium under conditions in which the cell expresses the recombinant protein; and (c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has a decreased level of

one or more of galactosylated glycans and fucosylated glycans relative to a corresponding level in a preparation of the recombinant protein produced by culturing the cell in the medium not comprising the reduced level of ammonium. In some embodiments, the culture medium comprises a reduced level of ammonium for a time and in an amount effective to increase the level of one or more of galactosylated glycans and fucosylated glycans of the recombinant protein. In some embodiments, the method further comprises processing, (e.g., one or more of formulating, filling into a container, labeling, packaging) the preparation into a drug product if the preparation meets a target value of one or more of galactosylated glycans and fucosylated glycans.

[0031] In some embodiments, the medium comprises less than about 50 mM, 45 mM, 40 mM, 35 mM, 30 mM, 25 mM, 20 mM, 15 mM, 10 mM, 5 mM, 1 mM, 0.5 mM, 0.1 mM, or less, ammonium.

[0032] In some embodiments, the method further comprises measuring a level of one or more of galactosylated glycans and fucosylated glycans in the recombinant protein preparation. In some embodiments, the method further comprises recording the level in a print or computer-readable medium, e.g., in a test report, Material Safety Data Sheet (MSDS), batch record, Certificate of Testing (CofT) or Certificate of Analysis (CofA).

[0033] In some embodiments, the increased level of the one or more of galactosylated glycans and fucosylated glycans is higher than the corresponding level by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, or more, of the corresponding level. In some embodiments, the increased level is at least about 70% to 100% fucosylated glycans, e.g., at least about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% fucosylated glycans. In some embodiments, the one or more fucosylated glycans comprise one or more of G1F glycans and G2F glycans. In some embodiments, the increased level is at least about 1% to about 95% galactosylated glycans.

[0034] In another aspect, the invention features a method of producing a recombinant therapeutic antibody preparation (e.g., abciximab, adalimumab, alemtuzumab, basiliximab, bevacizumab, certolizumab, cetuximab, daclizumab, eculizumab, efalizumab, gemtuzumab, ibritumomab, infliximab, muromonab-CD3, natalizumab, omalizumab, palivizumab, panitumumab, ranibizumab, rituximab, tositumomab, or trastuzumab), the method comprising:

(a) providing a target value (e.g., a predetermined pharmaceutical product specification or a quality control criterion for a pharmaceutical preparation, e.g., a Certificate of Analysis (CofA), a Certificate of Testing (CofT), or a Master Batch Record of a reference therapeutic antibody product) of one or more of high mannose glycans (e.g., HM3, HM4, HM5, HM6. HM7, HM8, HM9, or combinations thereof), galactosylated glycans, and fucosylated glycans; (b) providing a CHO cell genetically engineered to express a recombinant antibody; (c) culturing the cell in a culture medium comprising ammonium (e.g., at least about 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or more, ammonium, or about 1 mM to about 50 mM ammonium, about 10 mM to about 20 mM ammonium, about 11 mM to about 19 mM ammonium, about 12 mM to about 18 mM ammonium, about 13 mM to about 17 mM ammonium, or about 14 mM to about 16 mM ammonium) under conditions in which the cell expresses the recombinant antibody; (d) harvesting (e.g., purifying or isolating from the cell and/or culture medium) a preparation of the recombinant antibody produced by the cell; and (e) formulating (e.g., one or more of formulating, filling into a container, labeling, packaging) the preparation into a drug product if the preparation meets the target value of the one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans.

[0035] In another aspect, the invention features a method of producing a recombinant protein preparation having a target value of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans, the method comprising: (a) providing a cell genetically engineered to express a recombinant protein; (b) culturing the cell in a culture medium comprising (e.g., supplemented with) lysine under conditions in which the cell expresses the recombinant protein; and (c) harvesting (e.g., purifying or isolating from the cell and/or culture medium) a preparation of the recombinant protein produced by the cell, wherein the preparation has the target value of the one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans. In some embodiments, the culture medium comprises lysine for a time and in an amount effective to modify (e.g., increase or decrease) one or more of the high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans of the recombinant protein.

[0036] In some embodiments, the culture medium comprises at least about 1 g/L, 2 g/L, 3 g/L, 4 g/L, 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 11 g/L, 12 g/L, 13 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, 25 g/L, 26 g/L, 27 g/L, 28 g/L, 29 g/L, 30 g/L, or more, lysine. In some embodiments, the culture medium comprises at least about 1 g/L to about 30 g/L lysine, about 1 g/L to about 10 g/L lysine, about 1 g/L to about 9 g/L lysine, about 2 g/L to about 8 g/L lysine, about 3 g/L to about 7 g/L lysine, about 4 g/L to about 6 g/L lysine, about 10 g/L to about 20 g/L lysine, about 20 g/L to about 30 g/L lysine, about 1 g/L to about 20 g/L lysine, about 1 g/L to about 15 g/L lysine, or about 15 g/L lysine to about 30 g/L lysine. In some embodiments, the culture medium comprises at least about 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 110 mM, 120 mM, 130 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM, 200 mM, or more, lysine. In some embodiments, the culture medium comprises at least about 10 mM to about 200 mM lysine, about 10 mM to about 50 mM, lysine, about 50 mM to about 100 mM lysine, about 100 mM to about 150 mM lysine, about 150 mM to about 200 mM lysine, about10 mM to about 100 mM lysine, or about 100 mM to about 200 mM lysine.

[0037] In some embodiments, the target value is a level of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans in a reference therapeutic product. In some embodiments, the target value is a level of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans in a reference therapeutic antibody product. In some embodiments, the target value is a predetermined pharmaceutical product specification or a quality control criterion for a pharmaceutical preparation, e.g., a Certificate of Analysis (CofA), a Certificate of Testing (CofT), or a Master Batch Record. In some embodiments, the product specification is a product description in an FDA label, a Physician's Insert, a USP monograph, or an EP monograph.

[0038] In some embodiments, the reference therapeutic product is selected from the group consisting of: abatacept, abciximab, adalimumab, aflibercept, alefacept, alemtuzumab, basiliximab, bevacizumab, belatacept, certolizumab, cetuximab, daclizumab, eculizumab, efalizumab, entanercept, gemtuzumab, ibritumomab, infliximab, muromonab-CD3, natalizumab, omalizumab, palivizumab; panitumumab, ranibizumab, rilonacept, rituximab, tositumomab, and trastuzumab.

[0039] In some embodiments, the target value is one or more of: (a) at least about 0.1% to about 20% high mannose glycans, e.g., at least about 0.1%, about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, or more, high mannose glycans; (b) at least about 1% to about 95% galactosylated glycans, e.g., at least about 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% galactosylated glycans; (c) at least about 70% to 100% fucosylated glycans, e.g., at least about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% fucosylated glycans; and (d) at least about 0.1% to about 90% sialylated glycans, e.g., at least about 0.1%, 0.5%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% sialylated glycans. High mannose glycans can be, e.g., HM3, HM4, HM5, HM6, HM7, HM8, HM9, or combinations thereof.

[0040] In some embodiments, the target value of the one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans is higher than a corresponding level in a preparation produced by culturing the cell in the medium not comprising lysine, e.g., an elevated level of lysine. In some embodiments, the target value is higher than the corresponding level by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, or more, of the corresponding level.

[0041] In some embodiments, the method further comprises evaluating the level of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans in the recombinant protein preparation. In some embodiments, the method further comprises recording the level in a print or computer-readable medium, e.g., in a test report, Material Safety Data Sheet (MSDS), batch record, Certificate of Testing (CofT) or Certificate of Analysis (CofA).

[0042] In another aspect, the invention features a method of producing a recombinant protein preparation, the method comprising: (a) providing a target value of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans; (b) providing a cell genetically engineered to express a recombinant protein; (c) culturing the cell in a culture medium comprising (e.g., supplemented with) lysine under conditions in which the cell expresses the recombinant protein; (d) harvesting a preparation of the recombinant protein

produced by the cell; and (e) processing (e.g., one or more of formulating, filling into a container, labeling, packaging) the preparation into a drug product if the preparation meets the target value of the one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans. In some embodiments, the culture medium comprises lysine for a time and in an amount effective to modify (e.g., increase or decrease) one or more of the high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans of the recombinant protein.

[0043] In some embodiments, the method further comprises evaluating the level of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans in the recombinant protein preparation. In some embodiments, the method further comprises recording the level in a print or computer-readable medium, e.g., in a test report, Material Safety Data Sheet (MSDS), batch record, or Certificate of Testing (CofT) or Certificate of Analysis (CofA).

[0044] In some embodiments, the culture medium comprises at least about 1 g/L, 2 g/L, 3 g/L, 4 g/L, 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 11 g/L, 12 g/L, 13 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, 25 g/L, 26 g/L, 27 g/L, 28 g/L, 29 g/L, 30 g/L, or more, lysine. In some embodiments, the culture medium comprises at least about 1 g/L to about 30 g/L lysine, about 1 g/L to about 10 g/L lysine, about 1 g/L to about 9 g/L lysine, about 2 g/L to about 8 g/L lysine, about 3 g/L to about 7 g/L lysine, about 4 g/L to about 6 g/L lysine, about 10 g/L to about 20 g/L lysine, about 20 g/L to about 30 g/L lysine, about 1 g/L to about 20 g/L lysine, about 1 g/L to about 15 g/L lysine, or about 15 g/L lysine to about 30 g/L lysine. In some embodiments, the culture medium comprises at least about 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 110 mM, 120 mM, 130 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM, 200 mM, or more, lysine. In some embodiments, the culture medium comprises at least about 10 mM to about 200 mM lysine, about 10 mM to about 50 mM, lysine, about 50 mM to about 100 mM lysine, about 100 mM to about 150 mM lysine, about 150 mM to about 200 mM lysine, about10 mM to about 100 mM lysine, or about 100 mM to about 200 mM lysine.

[0045] In some embodiments, the target value is a level of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans in a reference

therapeutic product. In some embodiments, the target value is a level of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans in a reference therapeutic antibody product. In some embodiments, the target value is a predetermined pharmaceutical product specification or a quality control criterion for a pharmaceutical preparation, e.g., a Certificate of Analysis (CofA), a Certificate of Testing (CofT), or a Master Batch Record. In some embodiments, the product specification is a product description in an FDA label, a Physician's Insert, a USP monograph, or an EP monograph.

[0046] In some embodiments, the reference therapeutic product is selected from the group consisting of: abatacept, abciximab, adalimumab, aflibercept, alefacept, alemtuzumab, basiliximab, bevacizumab, belatacept, certolizumab, cetuximab, daclizumab, eculizumab, efalizumab, entanercept, gemtuzumab, ibritumomab, infliximab, muromonab-CD3, natalizumab, omalizumab, palivizumab; panitumumab, ranibizumab, rilonacept, rituximab, tositumomab, and trastuzumab.

[0047] In some embodiments, the target value is one or more of: (a) at least about 0.1% to about 20% high mannose glycans, e.g., at least about 0.1%, about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, or more, high mannose glycans; (b) at least about 1% to about 95% galactosylated glycans, e.g., at least about 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% galactosylated glycans; (c) at least about 70% to 100% fucosylated glycans, e.g., at least about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% fucosylated glycans; and (d) at least about 0.1% to about 90% sialylated glycans, e.g., at least about 0.1%, 0.5%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% sialylated glycans. High mannose glycans can be, e.g., HM3, HM4, HM5, HM6, HM7, HM8, HM9, or combinations thereof.

[0048] In some embodiments, the target value of the one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans is higher than a corresponding level in a preparation produced by culturing the cell in the medium not comprising lysine, e.g., an elevated level of lysine. In some embodiments, the target value is higher than the

corresponding level by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, or more, of the corresponding level.

[0049] In another aspect, the invention features a method of increasing a level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans in a recombinant protein preparation, the method comprising: (a) providing a cell genetically engineered to express a recombinant protein; (b) culturing the cell in a culture medium comprising an elevated level of lysine (e.g., supplemented with lysine) under conditions in which the cell expresses the recombinant protein; and (c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has an increased level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans relative to a corresponding level in a preparation of the recombinant protein produced by culturing the cell in the medium not comprising the elevated level of lysine. In some embodiments, the culture medium comprises the elevated level of lysine for a time and in an amount effective to increase one or more of the high mannose glycans, afucosylated glycans, and G0F glycans of the recombinant protein. In some embodiments, the method further comprises processing, (e.g., one or more of formulating, filling into a container, labeling, packaging) the preparation into a drug product if the preparation meets a target value of one or more of high mannose glycans, afucosylated glycans, and G0F glycans.

[0050] In some embodiments, the culture medium comprises at least about 1 g/L, 2 g/L, 3 g/L, 4 g/L, 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 11 g/L, 12 g/L, 13 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, 25 g/L, 26 g/L, 27 g/L, 28 g/L, 29 g/L, 30 g/L, or more, lysine. In some embodiments, the culture medium comprises at least about 1 g/L to about 30 g/L lysine, about 1 g/L to about 10 g/L lysine, about 1 g/L to about 9 g/L lysine, about 2 g/L to about 8 g/L lysine, about 3 g/L to about 7 g/L lysine, about 4 g/L to about 6 g/L lysine, about 10 g/L to about 20 g/L lysine, about 20 g/L to about 30 g/L lysine, about 1 g/L to about 20 g/L lysine, about 1 g/L to about 15 g/L lysine, or about 15 g/L lysine to about 30 g/L lysine. In some embodiments, the culture medium comprises at least about 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 110 mM, 120 mM, 130 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM, 200 mM, or more, lysine. In some embodiments, the culture medium comprises at least about 10 mM to about 200 mM lysine, about 10 mM to about 50 mM, lysine, about 50 mM to about 100 mM lysine, about 100

mM to about 150 mM lysine, about 150 mM to about 200 mM lysine, about10 mM to about 100 mM lysine, or about 100 mM to about 200 mM lysine.

[0051] In some embodiments, the method further comprises measuring a level of the one or more of high mannose glycans, afucosylated glycans, and G0F glycans in the recombinant protein preparation. In some embodiments, the method further comprises recording the level in a print or computer-readable medium, e.g., in a test report, Material Safety Data Sheet (MSDS), batch record, Certificate of Testing (CofT) or Certificate of Analysis (CofA).

[0052] In some embodiments, the increased level of the one or more of high mannose glycans, afucosylated glycans, and G0F glycans is higher than the corresponding level by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, or more, of the corresponding level. In some embodiments, the increased level is one or more of: (a) at least about 0.1% to about 20% high mannose glycans, e.g., at least about 0.1%, about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, or more, high mannose glycans; (b) at least about 10% to 100% afucosylated glycans, e.g., at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or about 100% afucosylated glycans; and (c) at least about 0.1% to about 90% G0F glycans, e.g., at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% G0F glycans. High mannose glycans can be, e.g., HM3, HM4, HM5, HM6, HM7, HM8, HM9, or combinations thereof.

[0053] In another aspect, the invention features a method of decreasing a level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans in a recombinant protein preparation, the method comprising: (a) providing a cell genetically engineered to express a recombinant protein; (b) culturing the cell in a culture medium comprising a reduced level of lysine under conditions in which the cell expresses the recombinant protein; and (c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has a decreased level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans relative to a corresponding level in a preparation of the recombinant protein produced by culturing the cell in the medium not comprising the reduced level of lysine. In some embodiments, the culture medium comprises the reduced level of lysine for a time and in an

amount effective to decrease a level of one or more of the high mannose glycans, afucosylated glycans, and G0F glycans of the recombinant protein. In some embodiments, the method further comprises processing, (e.g., one or more of formulating, filling into a container, labeling, packaging) the preparation into a drug product if the preparation meets a target value of one or more of high mannose glycans, afucosylated glycans, and G0F glycans.

[0054] In some embodiments, the medium comprises less than about 30 g/L, 29 g/L, 28 g/L, 27 g/L, 26 g/L, 25 g/L, 24 g/L, 23 g/L, 22 g/L, 21 g/L, 20 g/L, 19 g/L, 18 g/L, 17 g/L, 16 g/L, 15 g/L, 14 g/L, 13 g/L, 12 g/L, 11 g/L, 10 g/L, 9 g/L, 8 g/L, 7 g/L, 6 g/L, 5 g/L, 4 g/L, 3 g/L, 2 g/L, 1 g/L, 0.5 g/L, 0.25 g/L, 0.1 g/L, or no, lysine. In some embodiments, the medium comprises less than about 200 mM, 190 mM, 180 mM, 170 mM, 160 mM, 150 mM, 140 mM, 130 mM, 120 mM, 110 mM, 100 mM, 90 mM, 80 mM, 70 mM, 60 mM, 50 mM, 40 mM, 30 mM, 20 mM, 10 mM, 5 mM, 1 mM, or less, lysine.

[0055] In some embodiments, the method further comprises measuring a level of the one or more of high mannose glycans, afucosylated glycans, and G0F glycans in the recombinant protein preparation. In some embodiments, the method further comprises recording the level in a print or computer-readable medium, e.g., in a test report, Material Safety Data Sheet (MSDS), batch record, Certificate of Testing (CofT) or Certificate of Analysis (CofA).

[0056] In some embodiments, the decreased level of the one or more of high mannose glycans, afucosylated glycans, and G0F glycans is lower than the corresponding level by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, of the corresponding level. In some embodiments, the decreased level is one or more of: (a) less than about 20% high mannose glycans; (b) less than about 90% afucosylated glycans; and (c) less than about 90% G0F glycans. High mannose glycans can be, e.g., HM3, HM4, HM5, HM6, HM7, HM8, HM9, or combinations thereof.

[0057] In another aspect, the invention features a method of decreasing a level of one or more of fucosylated glycans, galactosylated glycans, and sialylated glycans in a recombinant protein preparation, the method comprising: (a) providing a cell genetically engineered to express a recombinant protein; (b) culturing the cell in a culture medium comprising an elevated level of lysine (e.g., supplemented with lysine) under conditions in which the cell expresses the recombinant protein; and (c) harvesting a preparation of the recombinant protein produced by the

cell, wherein the preparation has a decreased level of one or more of fucosylated glycans, galactosylated glycans, and sialylated glycans relative to a corresponding level in a preparation of the recombinant protein produced by culturing the cell in the medium not comprising the elevated level of lysine. In some embodiments, the culture medium comprises the elevated level of lysine for a time and in an amount effective to decrease the level of one or more of fucosylated glycans, galactosylated glycans, and sialylated glycans of the recombinant protein. In some embodiments, the method further comprises processing, (e.g., one or more of formulating, filling into a container, labeling, packaging) the preparation into a drug product if the preparation meets a target value of one or more of fucosylated glycans, galactosylated glycans, and sialylated glycans.

[0058] In some embodiments, the culture medium comprises at least about 1 g/L, 2 g/L, 3 g/L, 4 g/L, 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 11 g/L, 12 g/L, 13 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, 25 g/L, 26 g/L, 27 g/L, 28 g/L, 29 g/L, 30 g/L, or more, lysine. In some embodiments, the culture medium comprises at least about 1 g/L to about 30 g/L lysine, about 1 g/L to about 10 g/L lysine, about 1 g/L to about 9 g/L lysine, about 2 g/L to about 8 g/L lysine, about 3 g/L to about 7 g/L lysine, about 4 g/L to about 6 g/L lysine, about 10 g/L to about 20 g/L lysine, about 20 g/L to about 30 g/L lysine, about 1 g/L to about 20 g/L lysine, about 1 g/L to about 15 g/L lysine, or about 15 g/L lysine to about 30 g/L lysine. In some embodiments, the culture medium comprises at least about 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 110 mM, 120 mM, 130 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM, 200 mM, or more, lysine. In some embodiments, the culture medium comprises at least about 10 mM to about 200 mM lysine, about 10 mM to about 50 mM, lysine, about 50 mM to about 100 mM lysine, about 100 mM to about 150 mM lysine, about 150 mM to about 200 mM lysine, about10 mM to about 100 mM lysine, or about 100 mM to about 200 mM lysine.

[0059] In some embodiments, the method further comprises measuring a level of one or more of fucosylated glycans, galactosylated glycans, and sialylated glycans in the recombinant protein preparation. In some embodiments, the method further comprises recording the level in a print or computer-readable medium, e.g., in a test report, Material Safety Data Sheet (MSDS), batch record, Certificate of Testing (CofT) or Certificate of Analysis (CofA).

[0060] In some embodiments, the decreased level of the one or more of fucosylated glycans, galactosylated glycans, and sialylated glycans is lower than the corresponding level by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, of the corresponding level. In some embodiments, the decreased level is less than about 100% fucosylated glycans, e.g., less than about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, or 10%, fucosylated glycans. In some embodiments, the one or more fucosylated glycans comprise one or more of G1F and G2F glycans. In some embodiments, the decreased level is less than about 95% galactosylated glycans, e.g., less than about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 1%, or 0.5%, galactosylated glycans. In some embodiments, the decreased level is less than about 90% sialylated glycans, e.g., less than about 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 1%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1%, sialylated glycans.

[0061] In another aspect, the invention features a method of increasing a level of one or more of fucosylated glycans, galactosylated glycans, and sialylated glycans in a recombinant protein preparation, the method comprising: (a) providing a cell genetically engineered to express a recombinant protein; (b) culturing the cell in a culture medium comprising a reduced level of lysine under conditions in which the cell expresses the recombinant protein; and (c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has a decreased level of one or more of fucosylated glycans, galactosylated glycans, and sialylated glycans relative to a corresponding level in a preparation of the recombinant protein produced by culturing the cell in the medium not comprising the reduced level of lysine. In some embodiments, the culture medium comprises a reduced level of lysine for a time and in an amount effective to increase the level of one or more of fucosylated glycans, galactosylated glycans, and sialylated glycans of the recombinant protein. In some embodiments, the method further comprises processing, (e.g., one or more of formulating, filling into a container, labeling, packaging) the preparation into a drug product if the preparation meets a target value of one or more of fucosylated glycans, galactosylated glycans, and sialylated glycans.

[0062] In some embodiments, the medium comprises less than about 30 g/L, 29 g/L, 28 g/L, 27 g/L, 26 g/L, 25 g/L, 24 g/L, 23 g/L, 22 g/L, 21 g/L, 20 g/L, 19 g/L, 18 g/L, 17 g/L, 16 g/L, 15 g/L, 14 g/L, 13 g/L, 12 g/L, 11 g/L, 10 g/L, 9 g/L, 8 g/L, 7 g/L, 6 g/L, 5 g/L, 4 g/L, 3 g/L, 2 g/L, 1 g/L, 0.5 g/L, 0.25 g/L, 0.1 g/L, or no, lysine. In some embodiments, the medium comprises less than about 200 mM, 190 mM, 180 mM, 170 mM, 160 mM, 150 mM, 140 mM, 130 mM,

120 mM, 110 mM, 100 mM, 90 mM, 80 mM, 70 mM, 60 mM, 50 mM, 40 mM, 30 mM, 20 mM, 10 mM, 5 mM, 1 mM, or less, lysine.

[0063] In some embodiments, the method further comprises measuring a level of one or more of fucosylated glycans, galactosylated glycans, and sialylated glycans in the recombinant protein preparation. In some embodiments, the method further comprises recording the level in a print or computer-readable medium, e.g., in a test report, Material Safety Data Sheet (MSDS), batch record, Certificate of Testing (CofT) or Certificate of Analysis (CofA).

[0064] In some embodiments, the increased level of the one or more of fucosylated glycans, galactosylated glycans, and sialylated glycans is higher than the corresponding level by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, or more, of the corresponding level. In some embodiments, the increased level is at least about 70% to 100% fucosylated glycans, e.g., at least about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% fucosylated glycans. In some embodiments, the one or more fucosylated glycans comprise one or more of G1F glycans and G2F glycans. In some embodiments, the increased level is at least about 1% to about 95% galactosylated glycans, e.g., at least about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% galactosylated glycans. In some embodiments, the increased level is at least about 0.1% to 90% sialylated glycans, e.g., at least about 0.5%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, sialylated glycans.

[0065] In some aspects described herein, the culturing step comprises a first stage and a second stage. In some embodiments, the first stage comprises culturing the cell in a culture medium comprising a first level of ammonium and/or lysine, and the second stage comprises culturing the cell in the culture medium comprising a second level of ammonium and/or lysine. In some embodiments, the first level is a reduced level relative to the second level, and the second level is an elevated level relative to the first level. In some embodiments, the first level is an elevated level and the second level is a reduced level.

[0066] In some embodiments, the first stage comprises culturing the cell in the first level of ammonium and/or lysine for about 1 to about 8 days, e.g., 1-7, 1-6, 1-5 days. In some embodiments, the second stage comprises culturing the cell in the second level of ammonium and/or lysine for about 1 to about 12 days, e.g., 1-10, 1-9, 1-8, 1-7, 1-6 days. In some

embodiments, the first stage is a growth stage. In some embodiments, the second stage is a production stage.

[0067] In some aspects described herein, the culture medium further comprises one or more of cysteine, manganese, copper, cobalt, putrescine, a peptone, glucose, galactose, glucosamine, glutamine, a lipid (e.g., cholesterol), dextran sulfate, and DMSO.

[0068] In some aspects described herein, the cell is a mammalian cell. In some embodiments, the mammalian cell is a CHO (e.g., CHO-K1, DG44, CHO-DXB11, CHOK1SV, CHO-S) Vero, BHK, HeLa, COS, MDCK, or HEK-293 cell.

[0069] In some aspects described herein, the recombinant protein is a recombinant therapeutic product. In some embodiments, the recombinant protein is a recombinant therapeutic antibody product. In some embodiments, the recombinant protein is a recombinant therapeutic fusion protein. In some embodiments, the recombinant protein is abatacept, abciximab, adalimumab, aflibercept, alefacept, alemtuzumab, basiliximab, bevacizumab, belatacept, certolizumab, cetuximab, daclizumab, eculizumab, efalizumab, entanercept, gemtuzumab, ibritumomab, infliximab, muromonab-CD3, natalizumab, omalizumab, palivizumab; panitumumab, ranibizumab, rilonacept, rituximab, tositumomab, and trastuzumab.

[0070] In some aspects described herein, the conditions in which cells (e.g., mammalian cells) express recombinant proteins comprise (i) a medium having a pH of about 6, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, or about 8; (ii) a temperature of about 25°C, about 26°C, about 27°C, about 28°C, about 29°C, about 30°C, about 31°C, about 32°C, about 33°C, about 34°C, about 35°C, about 36°C, about 37 °C, about 38°C, about 39°C, or about 40°C; and/or (iii) a culture volume of about 100 mL, about 200 mL, about 300 mL, about 400 mL, about 500 mL, about 600 mL, about 700 mL, about 800 mL, about 900 mL, about 1 L, about 2 L, about 3 L, about 5 L, about 10 L, about 20 L, about 30 L, about 40 L, about 50 L, about 100 L, about 200 L, about 300 L, about 400 L, about 500 L, about 600 L, about 700 L, about 800 L, about 900 L, about 1000 L, 5,000 L, 10,000 L, 20,000 L, or more.

[0071] In another aspect, the invention features a preparation of a recombinant protein produced using a method described herein.

[0072] In another aspect, the invention features a method of producing a recombinant therapeutic antibody preparation (e.g., abciximab, adalimumab, alemtuzumab, basiliximab, bevacizumab, certolizumab, cetuximab, daclizumab, eculizumab, efalizumab, gemtuzumab, ibritumomab, infliximab, muromonab-CD3, natalizumab, omalizumab, palivizumab, panitumumab, ranibizumab, rituximab, tositumomab, or trastuzumab), the method comprising: (a) providing a target value (e.g., a predetermined pharmaceutical product specification or a quality control criterion for a pharmaceutical preparation, e.g., a Certificate of Analysis (CofA), a Certificate of Testing (CofT), or a Master Batch Record of a reference therapeutic antibody product) of one or more of high mannose glycans (e.g., HM3, HM4, HM5, HM6. HM7, HM8, HM9, or combinations thereof), galactosylated glycans, fucosylated glycans, and sialylated glycans; (b) providing a CHO cell genetically engineered to express a recombinant antibody; (c) culturing the cell in a culture medium comprising lysine (e.g., at least about 1 g/L, 2 g/L, 3 g/L, 4 g/L, 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 11 g/L, 12 g/L, 13 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, 25 g/L, 26 g/L, 27 g/L, 28 g/L, 29 g/L, 30 g/L, or more, lysine, or at least about 1 g/L to about 30 g/L lysine, about 1 g/L to about 30 g/L lysine, about 1 g/L to about 10 g/L lysine, about 1 g/L to about 9 g/L lysine, about 2 g/L to about 8 g/L lysine, about 3 g/L to about 7 g/L lysine, or about 4 g/L to about 6 g/L lysine), under conditions in which the cell expresses the recombinant antibody; (d) harvesting (e.g., purifying or isolating from the cell and/or culture medium) a preparation of the recombinant antibody produced by the cell; and (e) formulating (e.g., one or more of formulating, filling into a container, labeling, packaging) the preparation into a drug product if the preparation meets the target value of the one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans.

BRIEF DESCRIPTION OF THE DRAWINGS

[0073] The present teachings described herein will be more fully understood from the following description of various illustrative embodiments, when read together with the accompanying drawings. It should be understood that the drawings described below are for illustration purposes only and are not intended to limit the scope of the present teachings in any way.

[0074] FIG. 1 is a schematic illustration of an IgG antibody molecule.

[0075] FIG. 2A is a graphic representation of fucosylated glycan levels in preparations of a model antibody from cells grown in medium with or without ammonium feed. FIG. 2B is a graphic representation of G0F, G1F, and G2F glycan levels in preparations of a model antibody from cells grown in medium with or without ammonium feed. FIG. 2C is a graphic representation of afucosylated glycan levels in preparations of a model antibody from cells grown in medium with or without ammonium feed.

[0076] FIG. 3 is a graphic representation of sialylated glycan levels in preparations of a model antibody from cells grown in medium with or without ammonium feed.

[0077] FIG. 4A is a graphic representation of total high mannose glycan levels in preparations of a model antibody from cells grown in medium with or without ammonium feed. FIG. 4B is a graphic representation of high mannose 5 (HM5) glycan level in preparations of a model antibody from cells grown in medium with or without ammonium feed. FIG. 4C is a graphic representation of high mannose 6 (HM6), high mannose 8 (HM8), or high mannose 3 and 4 (HM3 and HM4) glycan level in preparations of a model antibody from cells grown in medium with or without ammonium feed.

[0078] FIG. 5A is a graphic representation of total fucosylated glycan levels in preparations of a model antibody from cells grown in medium with or without lysine feed. FIG. 5B is a graphic representation of G0F, G1F, and G2F glycan levels in preparations of a model antibody from cells grown in medium with or without lysine feed. FIG. 5C is a graphic representation of afucosylated glycan level in preparations of a model antibody from cells grown in medium with or without lysine feed.

[0079] FIG. 6 is a graphic representation of total sialylated glycan levels in preparations of a model antibody from cells grown in medium with or without lysine feed.

[0080] FIG. 7A is a graphic representation of total high mannose glycan levels in preparations of a model antibody from cells grown in medium with or without lysine feed. FIG. 7B is a graphic representation of high mannose 5 (HM5) glycan level in preparations of a model antibody from cells grown in medium with or without lysine feed. FIG. 7C is a graphic representation of high mannose 6 (HM6), high mannose 8 (HM8), or high mannose 3 and 4

(HM3 and HM4) glycan level in preparations of a model antibody from cells grown in medium with or without lysine feed.

DETAILED DESCRIPTION

[0081] The inventors have discovered that preparations of polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) can be produced from cells cultured in a medium having ammonium and/or lysine, e.g., an elevated level of ammonium and/or lysine effective to cause such effect. The present disclosure encompasses preparations of polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans), methods of making such polypeptides (e.g., antibodies), and methods of using such polypeptides (e.g., antibodies).

Definitions

[0082] As used herein, "purified" (or "isolated") refers to a nucleic acid sequence (e.g., a polynucleotide) or an amino acid sequence (e.g., a polypeptide) that is substantially free of other components. In some embodiments, a purified polynucleotide or purified polypeptide is removed or separated from other components present in its natural environment. For example, an isolated polypeptide is one that is separated from other components of a cell in which it was produced (e.g., the endoplasmic reticulum or cytoplasmic proteins and RNA). An isolated polynucleotide is one that is separated from other nuclear components (e.g., histones) and/or from upstream or downstream nucleic acid sequences. An isolated nucleic acid sequence or amino acid sequence can be at least 60% free, or at least 75% free, or at least 90% free, or at least 95% free from other components present in natural environment of the indicated nucleic acid sequence or amino acid sequence.

[0083] As used herein, "polynucleotide" (or "nucleotide sequence" or "nucleic acid molecule") refers to an oligonucleotide, nucleotide, or polynucleotide, and fragments or portions thereof, and to DNA and RNA of genomic or synthetic origin, which may be single- or double- stranded, and represent the sense or anti-sense strand.

[0084] As used herein, "polypeptide" (or "amino acid sequence" or "protein") refers to an oligopeptide, peptide, polypeptide, or protein sequence, and fragments or portions thereof, and to naturally occurring or synthetic molecules. "Amino acid sequence" and like terms, such as "polypeptide" or "protein", are not meant to limit the indicated amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

[0085] The term "pharmaceutically effective amount" or "therapeutically effective amount" refers to an amount (e.g., dose) effective in treating a patient, having a disorder or condition described herein. It is also to be understood herein that a "pharmaceutically effective amount" may be interpreted as an amount giving a desired therapeutic effect, either taken in one dose or in any dosage or route, taken alone or in combination with other therapeutic agents.

[0086] The term "treatment" or "treating", as used herein, refers to administering a therapy in an amount, manner, and/or mode effective to improve a condition, symptom, or parameter associated with a disorder or condition or to prevent or reduce progression of a disorder or condition, to a degree detectable to one skilled in the art. An effective amount, manner, or mode can vary depending on the subject and may be tailored to the subject.

[0087] As used herein, the term "antibody" refers to a polypeptide that includes at least one immunoglobulin variable region, e.g., an amino acid sequence that provides an immunoglobulin variable domain or immunoglobulin variable domain sequence. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as VH), and a light (L) chain variable region (abbreviated herein as VL). In another example, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions. The term "antibody" encompasses antigen-binding fragments of antibodies (e.g., single chain antibodies, Fab, $F(ab')_2$, Fd, Fv, and dAb fragments) as well as complete antibodies, e.g., intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof). The light chains of the immunoglobulin can be of types kappa or lambda. In some embodiments, an antibody includes an Fc region. In some embodiments, an antibody is a therapeutic antibody.

[0088] As used herein, the term "Fc region" refers to a dimer of two "Fc polypeptides", each "Fc polypeptide" comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. In some embodiments, an "Fc region" includes two Fc polypeptides linked by one or more disulfide bonds, chemical linkers, or peptide linkers. "Fc

polypeptide" refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and may also include part or all of the flexible hinge N-terminal to these domains. For IgG, "Fc polypeptide" comprises immunoglobulin domains Cgamma2 (Cγ2) and Cgamma3 (Cγ3) and the lower part of the hinge between Cgamma1 (Cγ1) and Cγ2. Although the boundaries of the Fc polypeptide may vary, the human IgG heavy chain Fc polypeptide is usually defined to comprise residues starting at T223 or C226 or P230, to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat et al. (1991, NIH Publication 91-3242, National Technical Information Services, Springfield, VA). For IgA, Fc polypeptide comprises immunoglobulin domains Calpha2 (Cα2) and Calpha3 (Cα3) and the lower part of the hinge between Calpha1 (Cα1) and Cα2. An Fc region can be synthetic, recombinant, or generated from natural sources such as IVIG.

[0089] As used herein, a "glycan" is a sugar. Glycans can be monomers or polymers of sugar residues, but typically contain at least three sugars, and can be linear or branched. A glycan may include natural sugar residues (e.g., glucose, N-acetylglucosamine, N-acetyl neuraminic acid, galactose, mannose, fucose, hexose, arabinose, ribose, xylose, etc.) and/or modified sugars (e.g., 2'-fluororibose, 2'-deoxyribose, phosphomannose, 6'-sulfo N-acetylglucosamine, etc). The term "glycan" includes homo and heteropolymers of sugar residues. The term "glycan" also encompasses a glycan component of a glycoconjugate (e.g., of a glycoprotein, glycolipid, proteoglycan, etc.). The term also encompasses free glycans, including glycans that have been cleaved or otherwise released from a glycoconjugate.

[0090] As used herein, a "high mannose glycan" refers to a glycan that includes at least 3 mannose sugar residues and that terminates in a mannose on a non-reducing end of the glycan. In some embodiments, a "high mannose glycan" includes at least 4, 5, 6, 7, 8, 9, 10, 11, or 12 mannose sugar residues.

[0091] As used herein, a "sialylated glycan" refers to a glycan that includes at least 1 sialic acid. In some embodiments, a sialylated glycan includes at least 1, 2, 3, or 4 sialic acids. In some embodiments, a sialylated glycan is a monosialylated glycan (e.g., a branched glycan monosialylated on an α1-3 arm of the branched glycan (e.g., with a NeuAc-α2,6-Gal terminal

linkage)), and/or a disialylated glycan (e.g., a branched glycan sialylated on both an α1-3 arm and an α1-6 arm of the branched glycan).

[0092] As used herein, a "galactosylated glycan" refers to a glycan that includes at least 1 galactose sugar residue. In some embodiments, a galactosylated glycan is a G1, G2, G1F, G2F, A1, and/or A2 glycan. In some embodiments, a galactosylated glycan includes one or more lactosamine repeats. In some embodiments, a galactosylated glycan is a galactose-alpha-1-3-galactose-containing glycan. In some embodiments, a galactosylated glycan is a tri-antennary glycan or a tetra-antennary glycan.

[0093] As used herein, the term "glycoprotein preparation" refers to a set of individual glycoprotein molecules, each of which comprises a polypeptide having a particular amino acid sequence (which amino acid sequence includes at least one glycosylation site) and at least one glycan covalently attached to the at least one glycosylation site. Individual molecules of a particular glycoprotein within a glycoprotein preparation typically have identical amino acid sequences but may differ in the occupancy of the at least one glycosylation sites and/or in the identity of the glycans linked to the at least one of the glycosylation sites. That is, a glycoprotein preparation may contain only a single glycoform of a particular glycoprotein, but more typically contains a plurality of glycoforms. Different preparations of the same glycoprotein may differ in the identity of glycoforms present (e.g., a glycoform that is present in one preparation may be absent from another) and/or in the relative amounts of different glycoforms.

[0094] The term "glycoform" is used herein to refer to a particular form of a glycoprotein. That is, when a glycoprotein includes a particular polypeptide that has the potential to be linked to different glycans or sets of glycans, then each different version of the glycoprotein (i.e., where the polypeptide is linked to a particular glycan or set of glycans) is referred to as a "glycoform".

[0095] "Reference glycoprotein", as used herein, refers to a glycoprotein having substantially the same amino acid sequence as (e.g., having about 95-100% identical amino acids of) a glycoprotein described herein, e.g., a glycoprotein to which it is compared. In some embodiments, a reference glycoprotein is a therapeutic glycoprotein described herein, e.g., an FDA approved therapeutic glycoprotein.

[0096] As used herein, an "N-glycosylation site of an Fc region" refers to an amino acid residue within an Fc region to which a glycan is N-linked.

[0097] "Target value", as used herein, refers to a predetermined level of one or more particular glycans, such as high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans. In some embodiments, a target value is an absolute value. In some embodiments, a target value is a relative value. In some embodiments, a target value is a level of one or more particular glycans, such as high mannose glycans (e.g., HM3, HM4, HM5, HM6, HM7, HM8, HM9, or combinations), fucosylated glycans (e.g., G0F, G1F, G2F, or combinations), galactosylated glycans (e.g., G1, G2, G1F, G2F, A1, A2 or combinations) and/or sialylated glycans (e.g., monosialylated, disialylated, or combinations), in a reference glycoprotein product or described in a specification or master batch record for a pharmaceutical product.

[0098] In some embodiments, a target value refers to an absolute level of (e.g., number of moles of) one or more glycans (e.g., high mannose glycans (e.g., one or more species of high mannose glycans), fucosylated glycans (e.g., one or more species of fucosylated glycans), galactosylated glycans (e.g., one or more species of galactosylated glycans), and/or sialylated glycans (e.g., one or more species of sialylated glycans) in a glycoprotein preparation. In some embodiments, a target value refers to a level of one or more glycans (e.g., high mannose glycans (e.g., one or more species of high mannose glycans), fucosylated glycans (e.g., one or more species of fucosylated glycans), galactosylated glycans (e.g., one or more species of galactosylated glycans), and/or sialylated glycans (e.g., one or more species of sialylated glycans) in a glycoprotein preparation relative to total level of glycans in the glycoprotein preparation. In some embodiments, a target value is expressed as a "percent", which refers to the number of moles of one or more glycans (e.g., Fc glycans) relative to total moles of glycans (e.g., Fc glycans) in a glycoprotein preparation. In some embodiments, "percent" refers to the number of moles of one or more PNGase F-released Fc glycans relative to total moles of PNGase F-released Fc glycans detected.

Cells

[0099] Any host cell that can be used to express a polypeptide of interest (e.g., an antibody) can be used in the methods described herein. The cells can be genetically engineered to contain a recombinant nucleic acid sequence, e.g., a gene, that encodes a polypeptide of interest (e.g., an antibody). For example, useful cells can express a recombinant polypeptide. Recombinant expression of a gene encoding a polypeptide can include construction of an expression vector containing a polynucleotide that encodes the polypeptide. Once a polynucleotide has been obtained, a vector for the production of the polypeptide can be produced by recombinant DNA technology using techniques known in the art. Known methods can be used to construct expression vectors containing polypeptide coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination.

[0100] An expression vector can be transferred to a host cell by conventional techniques, and the transfected cells can then be cultured by conventional techniques, modified in accordance with the present disclosure, to produce a recombinant polypeptide. A variety of host expression vector systems can be used (see, e.g., U.S. Pat. No. 5,807,715). Such host-expression systems (e.g., genetically engineered host expression systems) can be used to produce polypeptides (e.g., antibodies) and, where desired, subsequently purified. Such host expression systems include, but are not limited to, yeast (e.g., *Saccharomyces* and *Pichia*) transformed with recombinant yeast expression vectors containing polypeptide coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing polypeptide coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing polypeptide coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, NS0, and 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

[0101] For expression in mammalian host cells, viral-based expression systems can be utilized (see, e.g., Logan et al., 1984, Proc. Natl. Acad. Sci. USA 8:355-359). The efficiency of

expression can be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, e.g., Bittner et al., 1987, Methods in Enzymol. 153:516-544).

[0102] In addition, a host cell strain can be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the polypeptide (e.g., antibody) expressed. Such cells include, for example, established mammalian cell lines and insect cell lines, animal cells, fungal cells, and yeast cells. Mammalian host cells include, but are not limited to, CHO, Vero, BHK, HeLa, COS, MDCK, HEK-293, NIH-3T3, W138, BT483, Hs578T, HTB2, BT20, T47D, NS0 (a murine myeloma cell line that does not endogenously produce any immunoglobulin chains), CRL7O3O, HsS78Bst cells, PER.C6, SP2/0-Ag14, and hybridoma cells. Additional, nonlimiting examples of animal or mammalian host cells include Chinese hamster ovary cells (CHO), such as CHO-K1 (ATCC CCL-61), DG44 (Chasin et al., 1986, Som. Cell Molec. Genet., 12:555-556; and Kolkekar et al., 1997, Biochem., 36:10901-10909), CHO-DXB11 (G. Urlaub and L.A. Chasin, 1980 Proc. Natl. Acad. Sci., 77: 4216-4220 . L.H. Graf, and L.A. Chasin 1982, Molec. Cell. Biol., 2: 93-96), CHO-K1 Tet-On cell line (Clontech), CHO designated ECACC 85050302 (CAMR, Salisbury, Wiltshire, UK), CHO clone 13 (GEIMG, Genova, IT), CHO clone B (GEIMG, Genova, IT), CHO-K1/SF designated ECACC 93061607 (CAMR, Salisbury, Wiltshire, UK), RR-CHOK1 designated ECACC 92052129 (CAMR, Salisbury, Wiltshire, UK), CHOK1sv (Edmonds et al., Mol. Biotech. 34:179-190 (2006)), CHO-S (Pichler et al., Biotechnol. Bioeng. 108:386-94 (2011)), dihydrofolate reductase negative CHO cells (CHO/-DHFR, Urlaub and Chasin, 1980, Proc. Natl. Acad. Sci. USA, 77:4216), and dp12.CHO cells (U.S. Pat. No. 5,721,121); monkey kidney CV1 cells transformed by SV40 (COS cells, COS-7, ATCC CRL-1651); human embryonic kidney cells (e.g., 293 cells, or 293 cells subcloned for growth in suspension culture, Graham et al., 1977, J. Gen. Virol., 36:59); baby hamster kidney cells (BHK, ATCC CCL-10); monkey kidney cells (CV1, ATCC CCL-70); African green monkey kidney cells (VERO-76, ATCC CRL-1587; VERO, ATCC CCL-81); mouse sertoli cells (TM4, Mather, 1980, Biol. Reprod., 23:243-251); human cervical carcinoma cells (HELA, ATCC CCL-2); canine kidney cells (MDCK, ATCC CCL-34); human lung cells (W138, ATCC CCL-75); human hepatoma cells (HEP-G2, HB 8065); mouse mammary tumor

cells (MMT 060562, ATCC CCL-51); buffalo rat liver cells (BRL 3A, ATCC CRL-1442); TR1 cells (Mather, 1982, Ann. NY Acad. Sci., 383:44-68); MCR 5 cells; and FS4 cells.

[0103] For long-term, high-yield production of recombinant proteins, host cells can be engineered to stably express a polypeptide (e.g., antibody). Host cells can be transformed with DNA controlled by appropriate expression control elements known in the art, including promoter, enhancer, sequences, transcription terminators, polyadenylation sites, and selectable markers. Methods commonly known in the art of recombinant DNA technology can be used to select a desired recombinant clone. In some embodiments, a cell is genetically engineered to increase level of expression of an endogenous polypeptide, e.g., by increasing transcription of a gene encoding the polypeptide and/or increasing mRNA stability. In some embodiments, transcription of a gene encoding a polypeptide is increased by: altering the regulatory sequence of the endogenous gene, e.g., in a somatic cell, e.g., by the addition of a positive regulatory element, such as an enhancer or a DNA-binding site for a transcriptional activator; the deletion of a negative regulatory element, such as a DNA-binding site for a transcriptional repressor; and/or replacement of the endogenous regulatory sequence, or elements therein, with that of another gene, thereby allowing the coding region of the gene to be transcribed more efficiently.

[0104] Once a polypeptide described herein (e.g., an antibody described herein) has been produced by recombinant expression, it can be purified by any method known in the art for purification, for example, by chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. For example, an antibody can be isolated and purified by appropriately selecting and combining affinity columns such as Protein A column with chromatography columns, filtration, ultra filtration, salting-out and dialysis procedures (see Antibodies: A Laboratory Manual, Ed Harlow, David Lane, Cold Spring Harbor Laboratory, 1988). Further, as described herein, a polypeptide (e.g., an antibody) can be fused to heterologous polypeptide sequences to facilitate purification. Polypeptides having desired sugar chains can be separated with a lectin column by methods known in the art (see, e.g., WO 02/30954).

[0105] In accordance with the present disclosure, there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art.

Such techniques are described in the literature (see, e.g., Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; DNA Cloning: A Practical Approach, Volumes I and II (D. N. Glover ed. 1985); Oligonucleotide Synthesis (M. J. Gait ed. 1984); Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. (1985)); Transcription And Translation (B. D. Hames & S. J. Higgins, eds. (1984)); Animal Cell Culture (R. I. Freshney, ed. (1986)); Immobilized Cells and Enzymes (IRL Press, (1986)); B. Perbal, A Practical Guide To Molecular Cloning (1984); F. M. Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (1994).

Culture Methods

[0106] In general, polypeptides (e.g., antibodies) having target levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) can be produced by culturing cells in media that contains ammonium and/or lysine, e.g., an elevated, effective level of ammonium and/or lysine (e.g., during one or more stages of culture).

[0107] In some embodiments, cells genetically engineered to express a polypeptide are cultured (e.g., at one or more stages of culture) in a medium that includes ammonium and/or lysine, e.g., an elevated level of ammonium and/or lysine, to decrease levels of fucosylated glycans, G1F glycans, G2F glycans, and/or sialylated glycans in a preparation of the polypeptide expressed by the cells. In some embodiments, a level of fucosylated glycans, G1F glycans, G2F glycans, and/or sialylated glycans is decreased relative to the corresponding level(s) in a preparation of the polypeptide produced using the same medium that does not include ammonium and/or lysine, e.g., an elevated level of ammonium and/or lysine. In some embodiments, the decreased level of fucosylated glycans, G1F glycans, G2F glycans, and/or sialylated glycans is lower than the corresponding level(s) by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, of the corresponding level.

[0108] In some embodiments, cells genetically engineered to express a polypeptide are cultured (e.g., at one or more stages of culture) in a medium that includes ammonium and/or lysine, e.g., an elevated level of ammonium and/or lysine, to increase levels of high mannose glycans, afucosylated glycans, and/or G0F glycans in a preparation of the polypeptide expressed

by the cells. In some embodiments, a level of high mannose glycans, afucosylated glycans, and/or G0F glycans is increased relative to the corresponding level(s) in a preparation of the polypeptide produced using the same medium that does not include ammonium and/or lysine, e.g., an elevated level of ammonium and/or lysine. In some embodiments, the increased level of high mannose glycans, afucosylated glycans, and/or G0F glycans is higher than the corresponding level(s) by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, or more, of the corresponding level.

[0109] In some embodiments, cells genetically engineered to express a polypeptide are cultured (e.g., at one or more stages of culture) in a medium that includes a reduced level of ammonium and/or lysine, to increase levels of fucosylated glycans, G1F glycans, G2F glycans, and/or sialylated glycans in a preparation of the polypeptide expressed by the cells. In some embodiments, a level of fucosylated glycans, G1F glycans, G2F glycans, and/or sialylated glycans is increased relative to the corresponding level(s) in a preparation of the polypeptide produced using the same medium that does not include a reduced level of ammonium and/or lysine. In some embodiments, the increased level of fucosylated glycans, G1F glycans, G2F glycans, and/or sialylated glycans is higher than the corresponding level(s) by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, or more, of the corresponding level.

[0110] In some embodiments, cells genetically engineered to express a polypeptide are cultured (e.g., at one or more stages of culture) in a medium that includes a reduced level of ammonium and/or lysine, to decrease levels of high mannose glycans, afucosylated glycans, and/or G0F glycans in a preparation of the polypeptide expressed by the cells. In some embodiments, a level of high mannose glycans, afucosylated glycans, and/or G0F glycans is decreased relative to the corresponding level(s) in a preparation of the polypeptide produced using the same medium that does not include a reduced level of ammonium and/or lysine. In some embodiments, the decreased level of high mannose glycans, afucosylated glycans, and/or G0F glycans is lower than the corresponding level(s) by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, of the corresponding level.

[0111] As used herein, an "elevated level" of a component (e.g., ammonium and/or lysine) means a higher concentration of the component (e.g., ammonium and/or lysine) than is present in a standard medium, a starting medium, a medium used at one or more stages of culture, and/or in a medium in which cells are grown and/or a polypeptide is produced. In some embodiments, a component is not present in a standard and/or starting medium, a medium used at one or more other stages of culture, and/or in a medium in which cells are grown and/or a polypeptide is produced, and an "elevated level" is any amount of the component. A medium can include an elevated level of a component initially (i.e., at the start of a culture), and/or medium can be supplemented with the component to achieve an elevated level of the component at a particular time or times (e.g., at one or more stages) during culturing.

[0112] As used herein, a "reduced level" of a component (e.g., ammonium and/or lysine) means a lower concentration of the component (e.g., ammonium and/or lysine) than is present in a standard medium, a starting medium, a medium used at one or more stages of culture, and/or in a medium in which cells are grown and/or a polypeptide is produced. A medium can include a reduced level of a component initially (i.e., at the start of a culture), a medium can be diluted at a particular time or times (e.g., at one or more stages) during culturing to reduce the level of a component, and/or a medium can be replaced with a medium having a reduced level of a component at a particular time or times (e.g., at one or more stages) during culturing. In some embodiments, a reduced level of a component is no detectable level of the component in a medium.

[0113] In some embodiments, an elevated level of ammonium is an increase of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 550%, 600%, 650%, 700%, 750%, 800%, 850%, 900%, 950%, 1000% or more, relative to a level of ammonium in a standard medium, a starting medium, a medium during one or more stages of culture, and/or in a medium in which cells are grown and/or a polypeptide is produced.

[0114] In some embodiments, an elevated level of ammonium is an increase in level of ammonium by at least about 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or more, relative to a level of ammonium in a

standard medium, a starting medium, a medium during one or more stages of culture, and/or in a medium in which cells are grown and/or a polypeptide is produced.

[0115] In some embodiments, an elevated level of ammonium is about 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or more, ammonium. In some embodiments, an elevated level of ammonium is about 1 mM to about 50 mM ammonium, e.g., about 1 mM to about 10 mM ammonium, about 10 mM to about 20 mM ammonium, about 11 mM to about 19 mM ammonium, about 12 mM to about 18 mM ammonium, about 13 mM to about 17 mM ammonium, about 14 mM to about 16 mM ammonium, about 20 mM to about 30 mM ammonium, about 30 mM to about 40 mM ammonium, about 40 mM to about 50 mM ammonium, about 1 mM to about 25 mM ammonium, about 25 mM to about 50 mM ammonium, about 1 mM to about 20 mM ammonium, about 1 mM to about 30 mM ammonium, about 1 mM to about 40 mM ammonium, about 10 mM to about 50 mM ammonium, about 20 mM to about 50 mM ammonium, or about 30 mM to about 50 mM ammonium.

[0116] In some embodiments, a reduced level of ammonium is a decrease of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%, relative to a level of ammonium in a standard medium, a starting medium, a medium during one or more stages of culture, and/or in a medium in which cells are grown and/or a polypeptide is produced.

[0117] In some embodiments, a reduced level of ammonium is a decrease in level of ammonium by at least 1 mM, 5 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or more, relative to a level of ammonium in a standard medium, a starting medium, a medium during one or more stages of culture, and/or in a medium in which cells are grown and/or a polypeptide is produced.

[0118] In some embodiments, a reduced level of ammonium is less than about 50 mM, 45 mM, 40 mM, 35 mM, 30 mM, 25 mM, 20 mM, 15 mM, 10 mM, 5 mM, 1 mM, 0.5 mM, 0.1 mM, or less, ammonium.

[0119] In some embodiments, an elevated level of lysine is an increase of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 550%, 600%, 650%, 700%, 750%, 800%, 850%, 900%, 950%, 1000% or more,

relative to a level of lysine in a standard medium, a starting medium, a medium during one or more stages of culture, and/or in a medium in which cells are grown and/or a polypeptide is produced.

[0120] In some embodiments, an elevated level of lysine is an increase in level of lysine by at least about 0.1 g/L, 0.5 g/L, 1 g/L, 3 g/L, 4 g/L, 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 11 g/L, 12 g/L, 13 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, 25 g/L, 26 g/L, 27 g/L, 28 g/L, 29 g/L, 30 g/L, or more, relative to a level of lysine in a standard medium, a starting medium, a medium during one or more stages of culture, and/or in a medium in which cells are grown and/or a polypeptide is produced. In some embodiments, an elevated level of lysine is an increase in level of lysine by at least about 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 110 mM, 120 mM, 130 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM, 200 mM, or more, relative to a level of lysine in a standard medium, a starting medium, a medium during one or more stages of culture, and/or in a medium in which cells are grown and/or a polypeptide is produced.

[0121] In some embodiments, an elevated level of lysine is about 1 g/L, 2 g/L, 3 g/L, 4 g/L, 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 11 g/L, 12 g/L, 13 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, 25 g/L, 26 g/L, 27 g/L, 28 g/L, 29 g/L, 30 g/L, or more, lysine. In some embodiments, an elevated level of lysine is about 1 g/L to about 30 g/L lysine, about 1 g/L to about 10 g/L lysine, about 1 g/L to about 9 g/L lysine, about 2 g/L to about 8 g/L lysine, about 3 g/L to about 7 g/L lysine, about 4 g/L to about 6 g/L lysine, about 10 g/L to about 20 g/L lysine, about 20 g/L to about 30 g/L lysine, about 1 g/L to about 20 g/L lysine, about 1 g/L to about 15 g/L lysine, or about 15 g/L lysine to about 30 g/L lysine. In some embodiments, an elevated level of lysine is at least about 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 110 mM, 120 mM, 130 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM, 200 mM, or more, lysine. In some embodiments, an elevated level of lysine is at least about 10 mM to about 200 mM lysine, about 10 mM to about 50 mM, lysine, about 50 mM to about 100 mM lysine, about 100 mM to about 150 mM lysine, about 150 mM to about 200 mM lysine, about10 mM to about 100 mM lysine, or about 100 mM to about 200 mM lysine.

[0122] In some embodiments, a reduced level of lysine is a decrease of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%, relative to a level of lysine in a standard medium, a starting medium, a medium during one or more stages of culture, and/or in a medium in which cells are grown and/or a polypeptide is produced.

[0123] In some embodiments, a reduced level of lysine is a decrease in level of lysine by at least about 0.1 g/L, 0.5 g/L, 1 g/L, 3 g/L, 4 g/L, 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 11 g/L, 12 g/L, 13 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, 25 g/L, 26 g/L, 27 g/L, 28 g/L, 29 g/L, 30 g/L, or more, relative to a level of lysine in a standard medium, a starting medium, a medium during one or more stages of culture, and/or in a medium in which cells are grown and/or a polypeptide is produced. In some embodiments, an elevated level of lysine is a decrease in level of lysine by at least about 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 110 mM, 120 mM, 130 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM, 200 mM, or more, relative to a level of lysine in a standard medium, a starting medium, a medium during one or more stages of culture, and/or in a medium in which cells are grown and/or a polypeptide is produced.

[0124] In some embodiments, a reduced level of lysine is less than about 30 g/L, 29 g/L, 28 g/L, 27 g/L, 26 g/L, 25 g/L, 24 g/L, 23 g/L, 22 g/L, 21 g/L, 20 g/L, 19 g/L, 18 g/L, 17 g/L, 16 g/L, 15 g/L, 14 g/L, 13 g/L, 12 g/L, 11 g/L, 10 g/L, 9 g/L, 8 g/L, 7 g/L, 6 g/L, 5 g/L, 4 g/L, 3 g/L, 2 g/L, 1 g/L, 0.5 g/L, 0.25 g/L, 0.1 g/L, or no, lysine. In some embodiments, a reduced level of lysine is less than about 200 mM, 190 mM, 180 mM, 170 mM, 160 mM, 150 mM, 140 mM, 130 mM, 120 mM, 110 mM, 100 mM, 90 mM, 80 mM, 70 mM, 60 mM, 50 mM, 40 mM, 30 mM, 20 mM, 10 mM, 5 mM, 1 mM, or less, lysine.

[0125] Cells can be cultured in a variety of cell culture media known in the art, which are modified according to the disclosure to include ammonium and/or lysine, as described herein. Cell culture medium is understood by those of skill in the art to refer to a nutrient solution in which cells, such as animal or mammalian cells, are grown. A cell culture medium generally includes one or more of the following components: an energy source (e.g., a carbohydrate such as glucose); amino acids; vitamins; lipids or free fatty acids; and trace elements, e.g., inorganic compounds or naturally occurring elements in the micromolar range. Cell culture medium can also contain additional components, such as hormones and other growth factors (e.g., insulin,

transferrin, epidermal growth factor, serum, and the like); salts (e.g., calcium, magnesium and phosphate); buffers (e.g., HEPES); nucleosides and bases (e.g., adenosine, thymidine, hypoxanthine); antibiotics (e.g., gentamycin); and cell protective agents (e.g., a Pluronic polyol (Pluronic F68)).

[0126] In some embodiments, in addition to an elevated or reduced level of ammonium and/or lysine, a cell culture medium includes cysteine, manganese, copper, cobalt, putrescine, a peptone, glucose, galactose, galactosamine, glucosamine, glutamine, a lipid (e.g., cholesterol), dextran sulfate, and/or DMSO. For example, a culture medium can include about 0.1 g/L to about 1 g/L cysteine; about 0.01 mM to about 0.5 mM manganese; about 0.1 μM to about 0.5 mM copper; about 0.1 mg/L to about 30 mg/L cobalt; about 0.01 mg/L to about 5 mg/L putrescine; about 0.1 g/L to about 10 g/L glucose; about 0.5 g/L to about 30 g/L peptone, e.g., a non-animal derived peptone such as soy or cottonseed; about 1 μM to about 1 mM galactosamine; about 0.1 g/L to about 5 g/L glucosamine; about 0.01 g/L to about 0.1 g/L dextran sulfate; and/or about 0.1% to about 5% DMSO (e.g., about 0.1% to about 2% DMSO, about 0.5% to about 1% DMSO, about 1% to about 1.5% DMSO, about 0.6% to about 1.4% DMSO, about 0.7% to about 1.3% DMSO, about 0.8% to about 1.2% DMSO, about 0.9% to about 1.1% DMSO, about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, or about 2% DMSO, or more).

[0127] Media that has been prepared or commercially available can be modified according to the present disclosure for utilization in the methods described herein. Nonlimiting examples of such media include Minimal Essential Medium (MEM, Sigma, St. Louis, Mo.); Ham's F10 Medium (Sigma); Dulbecco's Modified Eagles Medium (DMEM, Sigma); RPM I-1640 Medium (Sigma); HyClone cell culture medium (HyClone, Logan, Utah); Power CHO2 (Lonza Inc., Allendale, NJ); and chemically-defined (CD) media, which are formulated for particular cell types, e.g., CD-CHO Medium (Invitrogen, Carlsbad, Calif.). Culture medium suitable for particular cells being cultured can be determined by a person of ordinary skill in the art without undue experimentation, and such medium can be altered according to the disclosure.

[0128] Cell culture conditions (including pH, O_2, CO_2, agitation rate and temperature) suitable for cellular production of polypeptides described herein (e.g., antibodies) are those that are

known in the art, such as conditions for batch, continuous, or fed-batch culturing of cells. For example, pH of cell culture medium is generally maintained at about 6.8 to about 7.2.

[0129] In some embodiments, cells are cultured in one or more stages, and cells can be cultured in medium having an elevated or reduced level of ammonium and/or lysine in one or more stages. For example, a culture method can include a first stage (e.g., using a medium having a reduced level of ammonium and/or lysine) and a second stage (e.g., using a medium having an elevated level of ammonium and/or lysine). In some embodiments, a culture method can include a first stage (e.g., using a medium having an elevated level of ammonium and/or lysine) and a second stage (e.g., using a medium having a reduced level of ammonium and/or lysine). In some embodiments, a culture method includes more than two stages, e.g., 3, 4, 5, 6, or more stages, and any stage can include medium having an elevated level of ammonium and/or lysine, or a reduced level of ammonium and/or lysine. The length of culture is not limiting. For example, a culture method can be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more days. In some embodiments, a culture method includes at least two stages. For example, a first stage can include culturing cells in medium having a reduced level of ammonium and/or lysine (e.g., for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more days), and a second stage can include culturing cells in medium having an elevated level of ammonium and/or lysine (e.g., for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more days).

[0130] In some embodiments, cells are cultured in an initial medium having a reduced level of ammonium for 5 days, and the medium is supplemented with ammonium to result in an elevated level of ammonium on day 6. In some embodiments, cells are cultured in an initial medium having a reduced level of lysine for 2 days, and the medium is supplemented with lysine to result in an elevated level of lysine on day 3.

[0131] In some embodiments, a first stage of culture is a growth stage. Generally, during a growth stage, a cell culture undergoes a period of exponential cell growth (the log phase) where cells are generally rapidly dividing. In some embodiments, cells are cultured under such conditions such that cell growth is maximized. The growth cycle and conditions for maximizing growth of host cells can be determined for a particular host cell by a person of ordinary skill in the art without undue experimentation. In some embodiments, cells are maintained in a growth stage for a period of between 1 and 10 days. In some embodiments, cells are cultured in a

medium having a reduced level of ammonium and/or lysine or an elevated level of ammonium and/or lysine for all or part of a growth stage.

[0132] In some embodiments, a second stage of culture is a production stage. Generally, during a production stage, cell growth has plateaued or is maintained at a near constant level. During a production stage, logarithmic cell growth has ended and polypeptide production is increased. During this period of time, a medium can generally be supplemented to support continued polypeptide production and to achieve a desired polypeptide product. In some embodiments, cells are maintained in a production stage for a period of between 1 and 10 days. In some embodiments, cells are cultured in a medium having a reduced level of ammonium and/or lysine or an elevated level of ammonium and/or lysine for all or part of a production stage.

[0133] In some embodiments, level of ammonium in a culture medium is elevated or reduced by altering a level of one or more ammonium salts, e.g., ammonium nitrate, ammonium carbonate, ammonium acetate, ammonium methanesulfonate, ammonium tolylsulfonate, ammonium chloride, ammonium bromide, ammonium sulfate, or ammonium phosphate. In some embodiments, a culture medium described herein is supplemented with one or more ammonium salts, e.g., during one or more stages of culture, or during one or more portions of a stage of a culture.

[0134] In some embodiments, level of lysine in a culture medium is elevated or reduced by altering a level of one or more of L-lysine, D-lysine, H-lysine, Z-lysine, hydroxylysine, and/or a lysine derivative.

[0135] In some embodiments, polypeptides (e.g., antibodies) having target levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) are produced by culturing cells in media that contains one or more agents capable of directly or indirectly modifying pH (e.g., intracellular pH of a cell described herein) ("pH modifying agent"). Such pH modifying agents include, without limitation, ammonium salts, basic amino acids (e.g., lysine, arginine, and/or histidine), polar amino acids (e.g., glutamine, asparagine, serine, or threonine), glucose, chloroquine, methylamine, tributylamine, benzylamine, and triethylamine. In some embodiments, cells are cultured in a culture medium having an elevated level or a reduced level of one or more pH modifying agents.

[0136] In some embodiments, cells genetically engineered to express a polypeptide are cultured (e.g., at one or more stages of culture) in a medium that includes an elevated level of chloroquine, methylamine, tributylamine, benzylamine, triethylamine, glucose, histidine, arginine, and/or glutamine, (i) to decrease levels of fucosylated glycans, G1F glycans, G2F glycans, and/or sialylated glycans; and/or (ii) to increase levels of G0F glycans, afucosylated glycans, and/or high mannose glycans, in a preparation of the polypeptide expressed by the cells.

[0137] In some embodiments, cells are cultured in a medium having an elevated level of chloroquine, e.g., about 10 μM to about 500 μM, e.g., about 10 μM, about 20 μM, about 30 μM, about 40 μM, about 50 μM, about 60 μM, about 70 μM, about 80 μM, about 90 μM, about 100 μM, about 110 μM, about 120 μM, about 130 μM, about 140 μM, about 150 μM, about 160 μM, about 170 μM, about 180 μM, about 190 μM, about 200 μM, about 210 μM, about 220 μM, about 230 μM, about 250 μM, about 250 μM, about 260 μM, about 270 μM, about 280 μM, about 290 μM, about 300 μM, about 310 μM, about 320 μM, about 330 μM, about 340 μM, about 350 μM, about 360 μM, about 370 μM, about 380 μM, about 390 μM, about 400 μM, about 410 μM, about 420 μM, about 430 μM, about 440 μM, about 450 μM, about 460 μM, about 470 μM, about 480 μM, about 490 μM, about 500 μM, or more.

[0138] In some embodiments, cells are cultured in a medium having an elevated level of methylamine, tributylamine, benzylamine, triethylamine, or a combination thereof, e.g., about 10 μM to about 3 mM, e.g., about 10 μM, about 50 μM, about 100 μM, about 150 μM, about 200 μM, about 250 μM, about 300 μM, about 350 μM, about 400 μM, about 450 μM, about 500 μM, about 550 μM, about 600 μM, about 650 μM, about 750 μM, about 800 μM, about 850 μM, about 900 μM, about 950 μM, about 1 mM, about 1.25 mM, about 1.5 mM, about 1.75 Mm, about 2 mM, about 2.25 mM, about 2.5 mM, about 2.75 mM, about 3 mM, or more.

[0139] In some embodiments, cells are cultured in a medium having an elevated level of glucose, e.g., about 0.1 g/L to about 10 g/L, e.g., about 0.2 g/L, about 0.3 g/L, about 0.4 g/L, about 0.5 g/L, about 0.6 g/L, about 0.7 g/L, about 0.8 g/L, about 0.9 g/L, about 1 g/L, about 1.1 g/L, about 1.2 g/L, about 1.3 g/L, about 1.4 g/L, about 1.5 g/L, about 1.6 g/L, about 1.7 g/L, about 1.8 g/L, about 1.9 g/L, about 2 g/L, about 2.1 g/L, about 2.2. g/L, about 2.3 g/L, about 2.4 g/L, about 2.5 g/L, about 2.6 g/L, about 2.7 g/L, about 2.8 g/L, about 2.9 g/L, about 3 g/L, about 3.5 g/L, about 4 g/L, about 4.5 g/L, about 5 g/L, about 5.5 g/L, about 6 g/L, about 6.5 g/L, about

7 g/L, about 7.5 g/L, about 8 g/L, about 8.5 g/L, about 9 g/L, about 9.5 g/L, about 10 g/L, or more.

[0140] In some embodiments, cells are cultured in a medium having an elevated level of histidine, arginine, or a combination of histidine and arginine, e.g., at a concentration of about 1 g/L to about 50 g/L, for example, at least about 1 g/L, at least about 1.5 g/L, at least about 2 g/L, at least about 2.5 g/L, at least about 3 g/L, at least about 3.5 g/L, at least about 4 g/L, at least about 4.5 g/L, at least about 5 g/L, at least about 5.5 g/L, at least about 6 g/L, at least about 6.5 g/L, at least about 7 g/L, at least about 7.5 g/L, at least about 8 g/L, at least about 8.5 g/L, at least about 9 g/L, at least about 9.5 g/L, at least about 10 g/L, at least about 10.5 g/L, at least about 11 g/L, at least about 11.5 g/L, at least about 12 g/L, at least about 12.5 g/L, at least about 13 g/L, at least about 13.5 g/L, at least about 14 g/L, at least about 14.5 g/L, at least about 15 g/L, at least about 15.5 g/L, at least about 16 g/L, at least about 16.5 g/L, at least about 17 g/L, at least about 17.5 g/L, at least about 18 g/L, at least about 18.5 g/L, at least about 19 g/L, at least about 19.5 g/L, at least about 20 g/L, at least about 25 g/L, at least about 30 g/L, at least about 35 g/L, at least about 40 g/L, at least about 45 g/L, at least about 50 g/L, or more.

[0141] In some embodiments, cells are cultured in a medium having an elevated level of glutamine, e.g., about 5 mM to about 80 mM, e.g., about 5 mM, about 10 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, or more.

[0142] In general, cell culture methods are classified as batch culture, continuous culture, and fed-batch culture. Any of these culture methods can be used to grow cells that produce polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans).

Batch Culture

[0143] In batch culture, a small amount of seed culture solution is added to a medium and cells are grown without any addition of a new medium or discharge of culture solution during culture. For the production of polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) using

batch culture, the medium comprises an elevated level or a reduced level of ammonium and/or lysine at an initial stage of cell culture.

[0144] In some embodiments, polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) are produced by batch culture of cells expressing the polypeptide in a medium having an elevated level of ammonium. In some embodiments, cells are cultured in a medium having at least about 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or more, ammonium. In some embodiments, cells are cultured in a medium having at least about 1 mM to about 50 mM ammonium, e.g., about 1 mM to about 10 mM ammonium, about 10 mM to about 20 mM ammonium, about 11 mM to about 19 mM ammonium, about 12 mM to about 18 mM ammonium, about 13 mM to about 17 mM ammonium, about 14 mM to about 16 mM ammonium, about 20 mM to about 30 mM ammonium, about 30 mM to about 40 mM ammonium, about 40 mM to about 50 mM ammonium, about 1 mM to about 25 mM ammonium, about 25 mM to about 50 mM ammonium, about 1 mM to about 20 mM ammonium, about 1 mM to about 30 mM ammonium, about 1 mM to about 40 mM ammonium, about 10 mM to about 50 mM ammonium, about 20 mM to about 50 mM ammonium, or about 30 mM to about 50 mM ammonium.

[0145] In some embodiments, polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) are produced by batch culture of cells expressing the polypeptide in a medium having a reduced level of ammonium. In some embodiments, cells are cultured in a medium having less than less than about 50 mM, 45 mM, 40 mM, 35 mM, 30 mM, 25 mM, 20 mM, 15 mM, 10 mM, 5 mM, 1 mM, 0.5 mM, 0.1 mM, or less, ammonium.

[0146] In some embodiments, polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) are produced by batch culture of cells expressing the polypeptide in a medium having an elevated level of lysine. In some embodiments, cells are cultured in a medium having at least about 1 g/L, 2 g/L, 3 g/L, 4 g/L, 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 11 g/L, 12 g/L, 13 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, 25 g/L, 26

g/L, 27 g/L, 28 g/L, 29 g/L, 30 g/L, or more, lysine. In some embodiments, cells are cultured in a medium having at least about 1 g/L to about 30 g/L lysine, about 1 g/L to about 10 g/L lysine, about 1 g/L to about 9 g/L lysine, about 2 g/L to about 8 g/L lysine, about 3 g/L to about 7 g/L lysine, about 4 g/L to about 6 g/L lysine, about 10 g/L to about 20 g/L lysine, about 20 g/L to about 30 g/L lysine, about 1 g/L to about 20 g/L lysine, about 1 g/L to about 15 g/L lysine, or about 15 g/L lysine to about 30 g/L lysine. In some embodiments, an elevated level of lysine is at least about 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 110 mM, 120 mM, 130 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM, 200 mM, or more, lysine. In some embodiments, an elevated level of lysine is at least about 10 mM to about 200 mM lysine, about 10 mM to about 50 mM, lysine, about 50 mM to about 100 mM lysine, about 100 mM to about 150 mM lysine, about 150 mM to about 200 mM lysine, about10 mM to about 100 mM lysine, or about 100 mM to about 200 mM lysine.

[0147] In some embodiments, polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) are produced by batch culture of cells expressing the polypeptide in a medium having a reduced level of lysine. In some embodiments, cells are cultured in a medium having less than about 30 g/L, 29 g/L, 28 g/L, 27 g/L, 26 g/L, 25 g/L, 24 g/L, 23 g/L, 22 g/L, 21 g/L, 20 g/L, 19 g/L, 18 g/L, 17 g/L, 16 g/L, 15 g/L, 14 g/L, 13 g/L, 12 g/L, 11 g/L, 10 g/L, 9 g/L, 8 g/L, 7 g/L, 6 g/L, 5 g/L, 4 g/L, 3 g/L, 2 g/L, 1 g/L, 0.5 g/L, 0.25 g/L, 0.1 g/L, or no, lysine. In some embodiments, cells are cultured in a medium having less than about 200 mM, 190 mM, 180 mM, 170 mM, 160 mM, 150 mM, 140 mM, 130 mM, 120 mM, 110 mM, 100 mM, 90 mM, 80 mM, 70 mM, 60 mM, 50 mM, 40 mM, 30 mM, 20 mM, 10 mM, 5 mM, 1 mM, or less, lysine.

Continuous Culture

[0148] Continuous culture is a culture method in which a medium is added and discharged continuously during culture. This continuous method includes perfusion culture. For example, in the production of polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) using continuous culture, level of ammonium and/or lysine in the medium can be adjusted at one or more stages of culture to result in an elevated level or a reduced level of ammonium and/or lysine. In certain methods, culture medium used at a first stage of culture does not include an

elevated level or a reduced level of ammonium and/or lysine, but at a particular time point during continuous culture (such as during all or part of a growth stage and/or a production stage), medium added during culture is elevated or reduced in the level of ammonium and/or lysine.

[0149] In some embodiments, polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) are produced by continuous culture of cells expressing the polypeptide in a medium having an elevated level of ammonium (e.g., during one or more stages of continuous culture). In some embodiments, cells are cultured, during one or more stages of continuous culture, in medium having at least about 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or more, ammonium. In some embodiments, cells are cultured, during one or more stages of continuous culture, in a medium having at least about 1 mM to about 50 mM ammonium, e.g., about 1 mM to about 10 mM ammonium, about 10 mM to about 20 mM ammonium, about 11 mM to about 19 mM ammonium, about 12 mM to about 18 mM ammonium, about 13 mM to about 17 mM ammonium, about 14 mM to about 16 mM ammonium, about 20 mM to about 30 mM ammonium, about 30 mM to about 40 mM ammonium, about 40 mM to about 50 mM ammonium, about 1 mM to about 25 mM ammonium, about 25 mM to about 50 mM ammonium, about 1 mM to about 20 mM ammonium, about 1 mM to about 30 mM ammonium, about 1 mM to about 40 mM ammonium, about 10 mM to about 50 mM ammonium, about 20 mM to about 50 mM ammonium, or about 30 mM to about 50 mM ammonium.

[0150] In some embodiments, cells are cultured, during one or more stages of continuous culture, in a medium having a reduced level of ammonium. In some embodiments, cells are cultured, during one or more stages of continuous culture, in a medium having less than less than about 50 mM, 45 mM, 40 mM, 35 mM, 30 mM, 25 mM, 20 mM, 15 mM, 10 mM, 5 mM, 1 mM, 0.5 mM, 0.1 mM, or less, ammonium.

[0151] In some embodiments, polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) are produced by continuous culture of cells expressing the polypeptide in a medium having an elevated level of lysine (e.g., during one or more stages of continuous culture). In

some embodiments, cells are cultured, during one or more stages of continuous culture, in a medium having at least about 1 g/L, 2 g/L, 3 g/L, 4 g/L, 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 11 g/L, 12 g/L, 13 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, 25 g/L, 26 g/L, 27 g/L, 28 g/L, 29 g/L, 30 g/L, or more, lysine. In some embodiments, cells are cultured, during one or more stages of continuous culture, in a medium having in a medium having at least about 1 g/L to about 30 g/L lysine, about 1 g/L to about 10 g/L lysine, about 1 g/L to about 9 g/L lysine, about 2 g/L to about 8 g/L lysine, about 3 g/L to about 7 g/L lysine, about 4 g/L to about 6 g/L lysine, about 10 g/L to about 20 g/L lysine, about 20 g/L to about 30 g/L lysine, about 1 g/L to about 20 g/L lysine, about 1 g/L to about 15 g/L lysine, or about 15 g/L lysine to about 30 g/L lysine. In some embodiments, cells are cultured, during one or more stages of continuous culture, in a medium having at least about 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 110 mM, 120 mM, 130 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM, 200 mM, or more, lysine. In some embodiments, cells are cultured, during one or more stages of continuous culture, in a medium having at least about 10 mM to about 200 mM lysine, about 10 mM to about 50 mM, lysine, about 50 mM to about 100 mM lysine, about 100 mM to about 150 mM lysine, about 150 mM to about 200 mM lysine, about10 mM to about 100 mM lysine, or about 100 mM to about 200 mM lysine.

[0152] In some embodiments, cells are cultured, during one or more stages of continuous culture, in a medium having a reduced level of lysine. In some embodiments, cells are cultured, during one or more stages of continuous culture, in a medium having less than about 30 g/L, 29 g/L, 28 g/L, 27 g/L, 26 g/L, 25 g/L, 24 g/L, 23 g/L, 22 g/L, 21 g/L, 20 g/L, 19 g/L, 18 g/L, 17 g/L, 16 g/L, 15 g/L, 14 g/L, 13 g/L, 12 g/L, 11 g/L, 10 g/L, 9 g/L, 8 g/L, 7 g/L, 6 g/L, 5 g/L, 4 g/L, 3 g/L, 2 g/L, 1 g/L, 0.5 g/L, 0.25 g/L, 0.1 g/L, or no, lysine. In some embodiments, cells are cultured, during one or more stages of continuous culture, in a medium having less than about 200 mM, 190 mM, 180 mM, 170 mM, 160 mM, 150 mM, 140 mM, 130 mM, 120 mM, 110 mM, 100 mM, 90 mM, 80 mM, 70 mM, 60 mM, 50 mM, 40 mM, 30 mM, 20 mM, 10 mM, 5 mM, 1 mM, or less, lysine.

Fed-batch Culture

[0153] Fed-batch culture is a method between batch culture and continuous culture. In a fed-batch culture, a cell culture is fed or supplemented continuously or sequentially during culture, but unlike continuous culture, discharge of culture solution is not carried out during culture. For example, for the production of polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) using fed-batch culture, medium added during one or more stages of culture can have an elevated level or a reduced level of ammonium and/or lysine.

[0154] In some embodiments, polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) are produced by adding medium (at one or more stages) to a fed batch culture of cells expressing the polypeptide sufficient to achieve (at one or more stages) an elevated level of ammonium. In some embodiments, at least about 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or more, ammonium is achieved (e.g., by adding or supplementing with ammonium, e.g., at one or more stages). In some embodiments, at least about 1 mM to about 50 mM ammonium, e.g., about 1 mM to about 10 mM ammonium, about 10 mM to about 20 mM ammonium, about 11 mM to about 19 mM ammonium, about 12 mM to about 18 mM ammonium, about 13 mM to about 17 mM ammonium, about 14 mM to about 16 mM ammonium, about 20 mM to about 30 mM ammonium, about 30 mM to about 40 mM ammonium, about 40 mM to about 50 mM ammonium, about 1 mM to about 25 mM ammonium, about 25 mM to about 50 mM ammonium, about 1 mM to about 20 mM ammonium, about 1 mM to about 30 mM ammonium, about 1 mM to about 40 mM ammonium, about 10 mM to about 50 mM ammonium, about 20 mM to about 50 mM ammonium, or about 30 mM to about 50 mM ammonium, is achieved.

[0155] In some embodiments, polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) are produced by adding medium (at one or more stages) to a fed batch culture of cells expressing the polypeptide sufficient to achieve (at one or more stages) a reduced level of ammonium. In some embodiments, less than less than about 50 mM, 45 mM, 40 mM, 35 mM,

30 mM, 25 mM, 20 mM, 15 mM, 10 mM, 5 mM, 1 mM, 0.5 mM, 0.1 mM, or less, ammonium in the culture medium is achieved (at one or more stages).

[0156] In some embodiments, polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) are produced by adding medium (at one or more stages) to a fed batch culture of cells expressing the polypeptide sufficient to achieve (at one or more stages) an elevated level of lysine. In some embodiments, at least about 1 g/L, 2 g/L, 3 g/L, 4 g/L, 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 11 g/L, 12 g/L, 13 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, 25 g/L, 26 g/L, 27 g/L, 28 g/L, 29 g/L, 30 g/L, or more, lysine in the culture medium is achieved (e.g., by adding or supplementing with lysine, e.g., at one or more stages). In some embodiments, at least about 1 g/L to about 30 g/L lysine, about 1 g/L to about 10 g/L lysine, about 1 g/L to about 9 g/L lysine, about 2 g/L to about 8 g/L lysine, about 3 g/L to about 7 g/L lysine, about 4 g/L to about 6 g/L lysine, about 10 g/L to about 20 g/L lysine, about 20 g/L to about 30 g/L lysine, about 1 g/L to about 20 g/L lysine, about 1 g/L to about 15 g/L lysine, or about 15 g/L lysine to about 30 g/L lysine, in the culture medium is achieved (e.g., by adding or supplementing with lysine, e.g., at one or more stages). In some embodiments, at least about 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 110 mM, 120 mM, 130 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM, 200 mM, or more, lysine in the culture medium is achieved (e.g., by adding or supplementing with lysine, e.g., at one or more stages). In some embodiments, at least about 10 mM to about 200 mM lysine, about 10 mM to about 50 mM, lysine, about 50 mM to about 100 mM lysine, about 100 mM to about 150 mM lysine, about 150 mM to about 200 mM lysine, about10 mM to about 100 mM lysine, or about 100 mM to about 200 mM lysine, in the culture medium is achieved (e.g., by adding or supplementing with lysine, e.g., at one or more stages).

[0157] In some embodiments, polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) are produced by adding medium (at one or more stages) to a fed batch culture of cells expressing the polypeptide sufficient to achieve (at one or more stages) a reduced level of lysine. In some embodiments, less than about 30 g/L, 29 g/L, 28 g/L, 27 g/L, 26 g/L, 25 g/L, 24 g/L, 23 g/L, 22 g/L, 21 g/L, 20 g/L, 19 g/L, 18 g/L, 17 g/L, 16 g/L, 15 g/L, 14 g/L, 13 g/L, 12 g/L, 11 g/L, 10 g/L, 9 g/L, 8 g/L, 7 g/L, 6 g/L, 5 g/L, 4 g/L, 3 g/L, 2 g/L, 1 g/L, 0.5 g/L, 0.25 g/L, 0.1

g/L, or no, lysine in the culture medium is achieved (at one or more stages). In some embodiments, less than about 200 mM, 190 mM, 180 mM, 170 mM, 160 mM, 150 mM, 140 mM, 130 mM, 120 mM, 110 mM, 100 mM, 90 mM, 80 mM, 70 mM, 60 mM, 50 mM, 40 mM, 30 mM, 20 mM, 10 mM, 5 mM, 1 mM, or less, lysine in the culture medium is achieved (at one or more stages).

[0158] According to the present disclosure, cell culture can be carried out under conditions for large or small scale production of polypeptides (e.g., antibodies), using culture vessels and/or culture apparatuses that are conventionally employed for animal or mammalian cell culture. For example, tissue culture dishes, T-flasks, shaker flasks, and spinner flasks can be used on a laboratory scale. For culturing on a larger scale (e.g., 1 L, 10 L, 100 L, 500 L, 5000 L, or more), a fluidized bed bioreactor, a hollow fiber bioreactor, a roller bottle culture, or a stirred tank bioreactor system can be used (e.g., as described in U.S. Pat. Nos. 7,541,164 and 7,332,303).

[0159] In particular methods, levels of one or more glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) in a preparation of polypeptides (e.g., antibodies) are monitored during one or more times (e.g., one or more stages) of cell culture, thereby allowing adjustment (e.g., increasing or decreasing the amount of ammonium and/or lysine in the culture) or possibly termination of the culture in order, for example, to achieve a target level of polypeptides (e.g., antibodies) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans).

Polypeptides

[0160] Described herein are therapeutic preparations of polypeptides (e.g., glycoproteins), and methods of making and using such preparations. Glycoproteins include, for example, any of a variety of hematologic agents (including, for instance, erythropoietin, blood-clotting factors, etc.), interferons, colony stimulating factors, antibodies, enzymes, and hormones. The identity of a particular glycoprotein is not intended to limit the present disclosure, and a therapeutic preparation described herein can include any glycoprotein of interest, e.g., a glycoprotein having an Fc region.

[0161] A glycoprotein described herein can include a target-binding domain that binds to a target of interest (e.g., binds to an antigen). For example, a glycoprotein, such as an antibody,

can bind to a transmembrane polypeptide (e.g., receptor) or ligand (e.g., a growth factor). Exemplary molecular targets (e.g., antigens) for glycoproteins described herein (e.g., antibodies) include CD proteins such as CD2, CD3, CD4, CD8, CD11, CD19, CD20, CD22, CD25, CD33, CD34, CD40, CD52; members of the ErbB receptor family such as the EGF receptor (EGFR, HER1, ErbB1), HER2 (ErbB2), HER3 (ErbB3) or HER4 (ErbB4) receptor; macrophage receptors such as CRIg; tumor necrosis factors such as TNFα or TRAIL/Apo-2; cell adhesion molecules such as LFA-1, Mac1, p150,95, VLA-4, ICAM-1, VCAM and αvβ3 integrin including either α or β subunits thereof (e.g., anti-CD11a, anti-CD18 or anti-CD11b antibodies); growth factors and receptors such as EGF, FGFR (e.g., FGFR3) and VEGF; IgE; cytokines such as IL1; cytokine receptors such as IL2 receptor; blood group antigens; flk2/flt3 receptor; obesity (OB) receptor; mpl receptor; CTLA-4; protein C; neutropilins; ephrins and receptors; netrins and receptors; slit and receptors; chemokines and chemokine receptors such as CCL5, CCR4, CCR5; amyloid beta; complement factors, such as complement factor D; lipoproteins, such as oxidized LDL (oxLDL); lymphotoxins, such as lymphotoxin alpha (LTa). Other molecular targets include Tweak, B7RP-1, proprotein convertase subtilisin/kexin type 9 (PCSK9), sclerostin, c-kit, Tie-2, c-fms, and anti-M1.

Reference Polypeptides

[0162] In some embodiments, described herein are therapeutic polypeptide (e.g., glycoprotein) having targeted levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans), where the targeted levels are the levels of glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) in a reference polypeptide product (e.g., glycoprotein product). Nonlimiting, exemplary reference glycoprotein products can include abatacept (Orencia®, Bristol-Myers Squibb), abciximab (ReoPro®, Roche), adalimumab (Humira®, Bristol-Myers Squibb), aflibercept (Eylea®, Regeneron Pharmaceuticals), alefacept (Amevive®, Astellas Pharma), alemtuzumab (Campath®, Genzyme/Bayer), basiliximab (Simulect®, Novartis), belatacept (Nulojix®, Bristol-Myers Squibb), belimumab (Benlysta®, GlaxoSmithKline), bevacizumab (Avastin®, Roche), canakinumab (Ilaris®, Novartis), brentuximab vedotin (Adcetris®, Seattle Genetics), certolizumab (CIMZIA®, UCB, Brussels, Belgium), cetuximab (Erbitux®, Merck-Serono), daclizumab (Zenapax®, Hoffmann-La Roche), denileukin diftitox (Ontak®, Eisai),

denosumab (Prolia®, Amgen; Xgeva®, Amgen), eculizumab (Soliris®, Alexion Pharmaceuticals), efalizumab (Raptiva®, Genentech), etanercept (Enbrel®, Amgen-Pfizer), gemtuzumab (Mylotarg®, Pfizer), golimumab (Simponi®, Janssen), ibritumomab (Zevalin®, Spectrum Pharmaceuticals), infliximab (Remicade®, Centocor), ipilimumab (Yervoy™, Bristol-Myers Squibb), muromonab (Orthoclone OKT3®, Janssen-Cilag), natalizumab (Tysabri®, Biogen Idec, Elan), ofatumumab (Arzerra®, GlaxoSmithKline), omalizumab (Xolair®, Novartis), palivizumab (Synagis®, MedImmune), panitumumab (Vectibix®, Amgen), ranibizumab (Lucentis®, Genentech), rilonacept (Arcalyst®, Regeneron Pharmaceuticals), rituximab (MabThera®, Roche), tocilizumab (Actemra®, Genentech; RoActemra, Hoffman-La Roche) tositumomab (Bexxar®, GlaxoSmithKline), and trastuzumab (Herceptin®, Roche).

[0163] In some embodiments, a level of one or more glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) in a reference polypeptide product is determined by analyzing one or more preparations (e.g., one or more lots) of the reference polypeptide. In some embodiments, a level of one or more glycans (e.g., high mannose glycans, fucosylated glycans, galactosylated glycans, and/or sialylated glycans) in a reference polypeptide product is a range of the one or more glycans in two or more preparations of the reference polypeptide (e.g., two or more lots of the reference polypeptide product). In some embodiments, a level of one or more glycans is a range (e.g., spanning a lowest level of the one or more glycans to a highest level of the one or more glycans) in two or more lots of the reference polypeptide product.

N-Linked Glycosylation

[0164] N-linked oligosaccharide chains are added to a protein in the lumen of the endoplasmic reticulum (see Molecular Biology of the Cell, Garland Publishing, Inc. (Alberts et al., 1994)). Specifically, an initial oligosaccharide (typically 14-sugar) is added to the amino group on the side chain of an asparagine residue contained within the target consensus sequence of Asn-X-Ser/Thr, where X may be any amino acid except proline. The structure of this initial oligosaccharide is common to most eukaryotes, and contains 3 glucose, 9 mannose, and 2 N-acetylglucosamine residues. This initial oligosaccharide chain can be trimmed by specific

glycosidase enzymes in the endoplasmic reticulum, resulting in a short, branched core oligosaccharide composed of two N-acetylglucosamine and three mannose residues.

[0165] N-glycans can be subdivided into three distinct groups called "high mannose type", "hybrid type", and "complex type", with a common pentasaccharide core (Man (alpha1,6)-(Man(alpha1,3))-Man(beta1,4)-GlcpNAc(beta 1,4)-GlcpNAc(beta 1,N)-Asn) occurring in all three groups.

[0166] After initial processing in the endoplasmic reticulum, the glycoprotein is transported to the Golgi where further processing may take place. If the glycan is transferred to the Golgi before it is completely trimmed to the core pentasaccharide structure, it results in a "high-mannose glycan".

[0167] Additionally or alternatively, one or more monosaccharides units of N-acetylglucosamine may be added to core mannose subunits to form a "complex glycan". Galactose may be added to N-acetylglucosamine subunits, and sialic acid subunits may be added to galactose subunits, resulting in chains that terminate with any of a sialic acid, a galactose or an N-acetylglucosamine residue. Additionally, a fucose residue may be added to an N-acetylglucosamine residue of the core oligosaccharide. Each of these additions is catalyzed by specific glycosyl transferases, known in the art.

[0168] Sialic acids are a family of 9-carbon monosaccharides with heterocyclic ring structures. They bear a negative charge via a carboxylic acid group attached to the ring as well as other chemical decorations including N-acetyl and N-glycolyl groups. The two main types of sialyl residues found in glycoproteins produced in mammalian expression systems are N-acetyl-neuraminic acid (NeuAc) and N-glycolylneuraminic acid (NeuGc). These usually occur as terminal structures attached to galactose (Gal) residues at the non-reducing termini of both N- and O-linked glycans. The glycosidic linkage configurations for these sialyl groups can be either α2,3 or α2,6.

[0169] "Hybrid glycans" comprise characteristics of both high-mannose and complex glycans. For example, one branch of a hybrid glycan may comprise primarily or exclusively mannose residues, while another branch may comprise N-acetylglucosamine, sialic acid, and/or galactose sugars.

N-linked Glycosylation in Antibodies

[0170] Antibodies are glycosylated at conserved, N-linked glycosylation sites in the Fc regions of immunoglobulin heavy chains. For example, each heavy chain of an IgG antibody has a single N-linked glycosylation site at Asn297 of the CH2 domain (see Jefferis, Nature Reviews 8:226-234 (2009)). IgA antibodies have N-linked glycosylation sites within the CH2 and CH3 domains, IgE antibodies have N-linked glycosylation sites within the CH3 domain, and IgM antibodies have N-linked glycosylation sites within the CH1, CH2, CH3, and CH4 domains (see Arnold et al., J. Biol. Chem. 280:29080-29087 (2005); Mattu et al., J. Biol. Chem. 273:2260-2272 (1998); Nettleton et al., Int. Arch. Allergy Immunol. 107:328-329 (1995)).

[0171] Each antibody isotype has a distinct variety of N-linked carbohydrate structures in the constant regions. For example, IgG has a single N-linked biantennary carbohydrate at Asn297 of the CH2 domain in each Fc polypeptide of the Fc region, which also contains the binding sites for C1q and FcγR (see Jefferis et al., Immunol. Rev. 163:59-76 (1998); and Wright et al., Trends Biotech 15:26-32 (1997)). For human IgG, the core oligosaccharide normally consists of $GlcNAc_2Man_3GlcNAc$, with differing numbers of outer residues. Variation among individual IgG can occur via attachment of galactose and/or galactose-sialic acid at one or both terminal GlcNAc or via attachment of a third GlcNAc arm (bisecting GlcNAc), and/or attachment of fucose.

Antibodies

[0172] The basic structure of an IgG antibody is illustrated in Figure 1. As shown in Figure 1, an IgG antibody consists of two identical light polypeptide chains and two identical heavy polypeptide chains linked together by disulphide bonds. The first domain located at the amino terminus of each chain is variable in amino acid sequence, providing antibody binding specificities found in each individual antibody. These are known as variable heavy (VH) and variable light (VL) regions. The other domains of each chain are relatively invariant in amino acid sequence and are known as constant heavy (CH) and constant light (CL) regions. As shown in Figure 1, for an IgG antibody, the light chain includes one variable region (VL) and one

constant region (CL). An IgG heavy chain includes a variable region (VH), a first constant region (CH1), a hinge region, a second constant region (CH2), and a third constant region (CH3). In IgE and IgM antibodies, the heavy chain includes an additional constant region (CH4).

[0173] Antibodies described herein can include, for example, monoclonal antibodies, polyclonal antibodies (e.g., IVIG), multispecific antibodies, human antibodies, humanized antibodies, camelized antibodies, chimeric antibodies, single-chain Fvs (scFv), disulfide-linked Fvs (sdFv), and anti-idiotypic (anti-Id) antibodies, and antigen-binding fragments of any of the above. Antibodies can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass.

[0174] The term "Fc fragment", as used herein, refers to one or more fragments of an Fc region that retains an Fc function and/or activity described herein, such as binding to an Fc receptor. Examples of such fragments include fragments that include an N-linked glycosylation site of an Fc region (e.g., an Asn297 of an IgG heavy chain or homologous sites of other antibody isotypes), such as a CH2 domain. The term "antigen binding fragment" of an antibody, as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. Examples of binding fragments encompassed within the term "antigen binding fragment" of an antibody include a Fab fragment, a $F(ab')_2$ fragment, a Fd fragment, a Fv fragment, a scFv fragment, a dAb fragment (Ward et al., (1989) Nature 341:544-546), and an isolated complementarity determining region (CDR). These antibody fragments can be obtained using conventional techniques known to those with skill in the art, and fragments can be screened for utility in the same manner as are intact antibodies.

[0175] Reference glycoproteins described herein (e.g., reference antibodies) or fragments thereof can be produced by any method known in the art for synthesizing glycoproteins (e.g., antibodies) (see, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Brinkman et al., 1995, J. Immunol. Methods 182:41-50; WO 92/22324; WO 98/46645). Chimeric antibodies can be produced using methods described in, e.g., Morrison, 1985, Science 229:1202, and humanized antibodies by methods described in, e.g., U.S. Pat. No. 6,180,370.

[0176] Additional reference antibodies described herein are bispecific antibodies and multivalent antibodies, as described in, e.g., Segal et al., J. Immunol. Methods 248:1-6 (2001); and Tutt et al., J. Immunol. 147: 60 (1991).

Glycoprotein Conjugates

[0177] The disclosure includes glycoproteins (or Fc regions or Fc fragments containing one or more N-glycosylation sites thereof) that are conjugated or fused to one or more heterologous moieties. Heterologous moieties include, but are not limited to, peptides, polypeptides, proteins, fusion proteins, nucleic acid molecules, small molecules, mimetic agents, synthetic drugs, inorganic molecules, and organic molecules. In some instances, a glycoprotein conjugate is a fusion protein that comprises a peptide, polypeptide, protein scaffold, scFv, dsFv, diabody, Tandab, or an antibody mimetic fused to an Fc region, such as a glycosylated Fc region. A fusion protein can include a linker region connecting an Fc region to a heterologous moiety (see, e.g., Hallewell et al. (1989), J. Biol. Chem. 264, 5260-5268; Alfthan et al. (1995), Protein Eng. 8, 725-731; Robinson & Sauer (1996)).

[0178] Exemplary, nonlimiting reference glycoprotein conjugate products include abatacept (Orencia®, Bristol-Myers Squibb), aflibercept (Eylea®, Regeneron Pharmaceuticals), alefacept (Amevive®, Astellas Pharma), belatacept (Nulojix®, Bristol-Myers Squibb), denileukin diftitox (Ontak®, Eisai), etanercept (Enbrel®, Amgen-Pfizer), and rilonacept (Arcalyst®, Regeneron Pharmaceuticals).

[0179] In some instances, a glycoprotein conjugate includes an Fc region (or an Fc fragment containing one or more N-glycosylations site thereof) conjugated to a heterologous polypeptide of at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids.

[0180] In some instances, a glycoprotein conjugate includes an Fc region (or an Fc fragment containing one or more N-glycosylation sites thereof) conjugated to one or more marker sequences, such as a peptide to facilitate purification. A particular marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, Calif., 91311). Other peptide tags useful for purification include, but are

not limited to, the hemagglutinin "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., 1984, Cell 37:767) and the "Flag" tag.

[0181] In other instances, a glycoprotein conjugate includes an Fc region (or Fc fragment containing one or more N-glycosylation sites thereof) conjugated to a diagnostic or detectable agent. Such fusion proteins can be useful for monitoring or prognosing development or progression of disease or disorder as part of a clinical testing procedure, such as determining efficacy of a particular therapy. Such diagnosis and detection can be accomplished by coupling a glycoprotein to detectable substances including, but not limited to, various enzymes, such as but not limited to horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such as, but not limited to, streptavidin/biotin and avidin/biotin; fluorescent materials, such as, but not limited to, umbelliferone, fluorescein, fluorescein isothiocynate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent materials, such as, but not limited to, luminol; bioluminescent materials, such as but not limited to, luciferase, luciferin, and aequorin; radioactive materials, such as but not limited to iodine (^{131}I, ^{125}I, ^{123}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115}In, ^{113}In, ^{112}In, ^{111}In), technetium (^{99}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{153}Gd, ^{159}Gd, ^{149}Pm, ^{140}La, ^{169}Yb, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, and ^{117}Sn; positron emitting metals using various positron emission tomographies, non-radioactive paramagnetic metal ions, and molecules that are radiolabelled or conjugated to specific radioisotopes.

[0182] Techniques for conjugating therapeutic moieties to antibodies are well known (see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56. (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987)).

Glycan Evaluation

[0183] In some embodiments, glycans of glycoproteins are analyzed by any available suitable method. In some instances, glycan structure and composition as described herein are analyzed,

for example, by one or more, enzymatic, chromatographic, mass spectrometry (MS), chromatographic followed by MS, electrophoretic methods, electrophoretic methods followed by MS, nuclear magnetic resonance (NMR) methods, and combinations thereof. Exemplary enzymatic methods include contacting a glycoprotein preparation with one or more enzymes under conditions and for a time sufficient to release one or more glycan(s) (e.g., one or more exposed glycan(s)). In some instances, the one or more enzymes include(s) PNGase F. Exemplary chromatographic methods include, but are not limited to, Strong Anion Exchange chromatography using Pulsed Amperometric Detection (SAX-PAD), liquid chromatography (LC), high performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC), thin layer chromatography (TLC), amide column chromatography, and combinations thereof. Exemplary mass spectrometry (MS) include, but are not limited to, tandem MS, LC-MS, LC-MS/MS, matrix assisted laser desorption ionisation mass spectrometry (MALDI-MS), Fourier transform mass spectrometry (FTMS), ion mobility separation with mass spectrometry (IMS-MS), electron transfer dissociation (ETD-MS), and combinations thereof. Exemplary electrophoretic methods include, but are not limited to, capillary electrophoresis (CE), CE-MS, gel electrophoresis, agarose gel electrophoresis, acrylamide gel electrophoresis, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blotting using antibodies that recognize specific glycan structures, and combinations thereof. Exemplary nuclear magnetic resonance (NMR) include, but are not limited to, one-dimensional NMR (1D-NMR), two-dimensional NMR (2D-NMR), correlation spectroscopy magnetic-angle spinning NMR (COSY-NMR), total correlated spectroscopy NMR (TOCSY-NMR), heteronuclear single-quantum coherence NMR (HSQC-NMR), heteronuclear multiple quantum coherence (HMQC-NMR), rotational nuclear overhauser effect spectroscopy NMR (ROESY-NMR), nuclear overhauser effect spectroscopy (NOESY-NMR), and combinations thereof.

[0184] In some instances, techniques described herein may be combined with one or more other technologies for the detection, analysis, and or isolation of glycans or glycoproteins. For example, in certain instances, glycans are analyzed in accordance with the present disclosure using one or more available methods (to give but a few examples, see Anumula, Anal. Biochem., 350(1):1, 2006; Klein et al., Anal. Biochem., 179:162, 1989; and/or Townsend, R.R. Carbohydrate Analysis" High Performance Liquid Chromatography and Capillary

Electrophoresis., Ed. Z. El Rassi, pp 181-209, 1995; WO2008/128216; WO2008/128220; WO2008/128218; WO2008/130926; WO2008/128225; WO2008/130924; WO2008/128221; WO2008/128228; WO2008/128227; WO2008/128230; WO2008/128219; WO2008/128222; WO2010/071817; WO2010/071824; WO2010/085251; WO2011/069056; and WO2011/127322, each of which is incorporated herein by reference in its entirety). For example, in some instances, glycans are characterized using one or more of chromatographic methods, electrophoretic methods, nuclear magnetic resonance methods, and combinations thereof. In some embodiments, glycans are analyzed by labeling with a fluorescent dye and measuring levels of fluorescence.

[0185] In some instances, methods for evaluating one or more target protein specific parameters, e.g., in a glycoprotein preparation, e.g., one or more of the parameters disclosed herein, can be performed by one or more of the methods listed in Table 1.

Table 1: Exemplary methods of evaluating parameters:

Method(s)	**Relevant literature**	**Parameter**
C18 UPLC Mass Spec.*	Chen and Flynn, Anal. Biochem., 370:147-161 (2007) Chen and Flynn, J. Am. Soc. Mass Spectrom., 20:1821-1833 (2009)	Glycan(s) (e.g., N-linked glycan, exposed N-linked glycan, glycan detection, glycan identification, and characterization; site specific glycation; glycoform detection (e.g., parameters 1-7); percent glycosylation; and/or aglycosyl)
Bioanalyzer (reducing/non-reducing)*	Forrer et al., Anal. Biochem., 334:81-88 (2004)	Glycan (e.g., N-linked glycan, exposed N-linked glycan) (including, for example, glycan detection, identification, and characterization; site specific glycation; glycoform detection;

		percent glycosylation; and/or aglycosyl)
LC-MS (reducing/non-reducing/alkylated)* * Methods include removal (e.g., enzymatic, chemical, and physical) of glycans	Dick et al., Biotechnol. Bioeng., 100:1132-1143 (2008) Goetze et al., Glycobiol., 21:949-959 (2011) Xie et al., mAbs, 2:379-394 (2010)	Glycan (e.g., N-linked glycan, exposed N-linked glycan) (including, for example, glycan detection, identification, and characterization; site specific glycation; glycoform detection; percent glycosylation; and/or aglycosyl)
Anion-exchange chromatography	Ahn et al., J. Chrom. B, 878:403-408 (2010)	Sialylated glycan
1,2-diamino-4,5-methylenedioxybenzene (DMB) labeling method	Hokke et al., FEBS Lett., 275:9-14 (1990)	Sialic acid

The literature recited above are hereby incorporated by reference in their entirety or, in the alternative, to the extent that they pertain to one or more of the methods for determining a parameter described herein.

Pharmaceutical Compositions and Administration

[0186] A glycoprotein described herein can be incorporated (e.g., formulated) into a pharmaceutical composition. Such a pharmaceutical composition is useful as an alternative and/or improved composition for the prevention and/or treatment of one or more diseases relative to a corresponding reference glycoprotein. Pharmaceutical compositions comprising a glycoprotein can be formulated by methods known to those skilled in the art. The pharmaceutical composition can be administered parenterally in the form of an injectable formulation comprising a sterile solution or suspension in water or another pharmaceutically

acceptable liquid. For example, the pharmaceutical composition can be formulated by suitably combining the glycoprotein with pharmaceutically acceptable vehicles or media, such as sterile water and physiological saline, vegetable oil, emulsifier, suspension agent, surfactant, stabilizer, flavoring excipient, diluent, vehicle, preservative, binder, followed by mixing in a unit dose form required for generally accepted pharmaceutical practices. The amount of active ingredient included in the pharmaceutical preparations is such that a suitable dose within the designated range is provided.

[0187] A sterile composition for injection can be formulated in accordance with conventional pharmaceutical practices using distilled water for injection as a vehicle. For example, physiological saline or an isotonic solution containing glucose and other supplements such as D-sorbitol, D-mannose, D-mannitol, and sodium chloride may be used as an aqueous solution for injection, optionally in combination with a suitable solubilizing agent, for example, alcohol such as ethanol and polyalcohol such as propylene glycol or polyethylene glycol, and a nonionic surfactant such as polysorbate 80™, HCO-50 and the like.

[0188] Nonlimiting examples of oily liquid include sesame oil and soybean oil, and it may be combined with benzyl benzoate or benzyl alcohol as a solubilizing agent. Other items that may be included are a buffer such as a phosphate buffer, or sodium acetate buffer, a soothing agent such as procaine hydrochloride, a stabilizer such as benzyl alcohol or phenol, and an antioxidant. A formulated injection can be packaged in a suitable ampule.

[0189] In some instances, a level of one or more glycans described herein can be compared to a predetermined level (e.g., a corresponding level in a reference standard), e.g., to make a decision regarding the composition of the polypeptide preparation, e.g., a decision to classify, select, accept or discard, release or withhold, process into a drug product, ship, move to a different location, formulate, label, package, release into commerce, or sell or offer for sale the polypeptide, e.g., a recombinant antibody. In other instances, the decision can be to accept, modify or reject a production parameter or parameters used to make the polypeptide, e.g., an antibody. Particular, nonlimiting examples of reference standards include a control level (e.g., a polypeptide produced by a different method) or a range or value in a product specification (e.g., an FDA label or Physician's Insert) or quality criterion for a pharmaceutical preparation containing the polypeptide preparation.

[0190] In some instances, methods (i.e., evaluation, identification, and production methods) include taking action (e.g., physical action) in response to the methods disclosed herein. For example, a polypeptide preparation is classified, selected, accepted or discarded, released or withheld, processed into a drug product, shipped, moved to a different location, formulated, labeled, packaged, released into commerce, or sold or offered for sale, depending on whether the preselected or target value is met. In some instances, processing may include formulating (e.g., combining with pharmaceutical excipients), packaging (e.g., in a syringe or vial), labeling, or shipping at least a portion of the polypeptide preparation. In some instances, processing includes formulating (e.g., combining with pharmaceutical excipients), packaging (e.g., in a syringe or vial), and labeling at least a portion of the preparation as a drug product described herein. Processing can include directing and/or contracting another party to process as described herein.

[0191] In some instances, a biological activity of a polypeptide preparation (e.g., an antibody preparation) is assessed. Biological activity of the preparation can be analyzed by any known method. In some embodiments, a binding activity of a polypeptide is assessed (e.g., binding to a receptor). In some embodiments, a therapeutic activity of a polypeptide is assessed (e.g., an activity of a polypeptide in decreasing severity or symptom of a disease or condition, or in delaying appearance of a symptom of a disease or condition). In some embodiments, a pharmacologic activity of a polypeptide is assessed (e.g., bioavailability, pharmacokinetics, pharmacodynamics). For methods of analyzing bioavailability, pharmacokinetics, and pharmacodynamics of glycoprotein therapeutics, see, e.g., Weiner et al., J. Pharm. Biomed. Anal. 15(5):571-9, 1997; Srinivas et al., J. Pharm. Sci. 85(1):1-4, 1996; and Srinivas et al., Pharm. Res. 14(7):911-6, 1997.

[0192] The particular biological activity or therapeutic activity that can be tested will vary depending on the particular polypeptide (e.g., antibody). The potential adverse activity or toxicity (e.g., propensity to cause hypertension, allergic reactions, thrombotic events, seizures, or other adverse events) of polypeptide preparations can be analyzed by any available method. In some embodiments, immunogenicity of a polypeptide preparation is assessed, e.g., by determining whether the preparation elicits an antibody response in a subject.

[0193] Route of administration can be parenteral, for example, administration by injection, transnasal administration, transpulmonary administration, or transcutaneous administration.

Administration can be systemic or local by intravenous injection, intramuscular injection, intraperitoneal injection, subcutaneous injection.

[0194] A suitable means of administration can be selected based on the age and condition of the patient. A single dose of the pharmaceutical composition containing a modified glycoprotein can be selected from a range of 0.001 to 1000 mg/kg of body weight. On the other hand, a dose can be selected in the range of 0.001 to 100000 mg/body weight, but the present disclosure is not limited to such ranges. The dose and method of administration varies depending on the weight, age, condition, and the like of the patient, and can be suitably selected as needed by those skilled in the art.

[0195] All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described herein.

[0196] The disclosure is further illustrated by the following example. The example is provided for illustrative purposes only. It is not to be construed as limiting the scope or content of the disclosure in any way.

EXAMPLES

Example 1 - Effect of Ammonium on Glycosylation

Methods

[0197] The effect of ammonium on antibody glycoforms of a model antibody produced by CHO cells was analyzed. CHO cells genetically engineered to express the model antibody were grown initially in base media (Power CHO2, Catalog # BE15-771, Lonza Inc., Allendale, NJ) containing soy hydrolysate, Lonza Power Feed A and additional supplements. On Day 3, the cells were fed with Lonza Power Feed A and soy hydrolysate. On Day 6, the cells were fed with Lonza Power Feed A medium and additional supplements, with or without NH_4Cl (10 mM final

concentration). Cells were fed 2 g/L glucose when the glucose concentration dropped below 2 g/L. Cells were harvested on Day 14, and the antibodies produced were evaluated for titer and glycan composition.

[0198] Relative quantitation of each glycoform was performed on glycans enzymatically released from the antibody using PNGase F and labeled with the fluorophore 2-aminobenzamide. The relative quantities were based on the fluorescence intensity of the relevant species relative to the total fluorescence intensity in the chromatogram. All glycoforms eluting at the same retention time were considered together.

Results

[0199] The presence or absence of ammonium in the culture medium had minimal impact on titer or maximum VCD. Cells grown in the presence of ammonium had an average maximum VCD of 8 x 10^6 cells/mL and a titer of 1.0 g/L, whereas cells grown in the absence of ammonium had an average maximum VCD of 7.5 x 10^6 cells/mL and a titer of 1.1 g/L.

[0200] As shown in Figure 2A, the level of fucosylated glycans was decreased in antibodies grown in the presence of ammonium, while the level of afucosylated glycans was increased in antibodies grown in the presence of ammonium (see Figure 2C). Further, as shown in Figure 2B, the presence of ammonium resulted in a slight increase in the level of G0F glycans, whereas the levels of G1F/G2F glycans (which account for about 20-30% of fucosylated species) decreased in the presence of ammonium.

[0201] Ammonium also resulted in a decrease in the level of sialylated glycans (as shown in Figure 3).

[0202] The impact of ammonium on high mannose glycans is shown in Figures 4A, 4B, and 4C. As shown in Figure 4A, ammonium increased the total level of high mannose glycans compared to no ammonium. Further, ammonium led to increased levels of high mannose 5 glycans (Figure 4B), as well as high mannose 6 glycans and high mannose 3/4 glycans (see Figure 4C).

Example 2 - Effect of Lysine on Glycosylation

Method

[0203] To investigate the effect of lysine feed on antibody glycoforms of a model antibody, CHO cells genetically engineered to express the antibody were grown initially in base media (Power CHO2, Catalog # BE15-771, Lonza Inc., Allendale, NJ) containing soy hydrolysate, Lonza Power Feed A and additional supplements (and included an initial lysine concentration of about 800-950 mg/L). On Day 3, the cells were fed with Lonza Power Feed A and soy hydrolysate, without or with L-lysine (Catalog # LL501, Sigma) (5 g/L final concentration, "lysine feed"). On Day 6, the cells were fed with Lonza Power Feed A medium and additional supplements. Cells were fed 2 g/L glucose when the glucose concentration dropped below 2 g/L.

[0204] Cells were harvested on Day 14, and the antibodies produced were evaluated for titer and glycan composition. Relative quantitation of each glycoform was performed as described in Example 1.

Results

[0205] The presence or absence of lysine feed had minimal impact on titer or maximum VCD. Cells grown in the presence of lysine feed had an average maximum VCD of 8.0×10^6 cells/mL and a titer of 1.0 g/L whereas cells grown in the absence of a lysine feed had an average maximum VCD of 8.4×10^6 cells/mL and a titer of 0.9 g/L.

[0206] Lysine feed affected the level of fucosylated glycans, resulting in a decreased level of fucosylated glycans (Figure 5A), with an increase in afucosylated glycans (Figure 5C). Lysine feed resulted in increased level of G0F glycans and decreased levels of G1F/G2F glycans (Figure 5B). Further, lysine feed resulted in a decrease in sialylated glycans (Figure 6).

[0207] Lysine feed also increased the level of high mannose glycans (see Figure 7A), with an increase in high mannose 5 glycans (Figure 7B) as well as high mannose 6 glycans, high mannose 8 glycans, and high mannose 3/4 glycans (Figure 7C).

[0208] These Examples demonstrate that polypeptides having targeted levels of glycans can be produced using cells cultured in medium containing or lacking ammonium and/or lysine.

EQUIVALENTS

[0209] It is to be understood that while the disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

CLAIMS

1. A method of producing a recombinant protein preparation having a target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans, the method comprising:

(a) providing a cell genetically engineered to express a recombinant protein;

(b) culturing the cell in a culture medium comprising ammonium under conditions in which the cell expresses the recombinant protein; and

(c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has the target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans.

2. The method of claim 1, wherein the recombinant protein is a recombinant therapeutic protein.

3. The method of claim 1 or claim 2, wherein the recombinant protein is a recombinant therapeutic antibody.

4. The method of any one of claims 1-3, wherein the target value is a level of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans in a reference therapeutic product.

5. The method of any one of claims 1-3, wherein the target value is a level of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans in a reference therapeutic antibody product.

6. The method of any one of claims 1-3, wherein the target value is a predetermined pharmaceutical product specification.

7. The method of claim 4, wherein the reference therapeutic product is selected from the group consisting of: abatacept, abciximab, adalimumab, aflibercept, alefacept, alemtuzumab, basiliximab, bevacizumab, belatacept, certolizumab, cetuximab, daclizumab, eculizumab,

efalizumab, entanercept, gemtuzumab, ibritumomab, infliximab, muromonab-CD3, natalizumab, omalizumab, palivizumab; panitumumab, ranibizumab, rilonacept, rituximab, tositumomab, and trastuzumab.

8. The method of any one of claims 1-7, further comprising evaluating a level of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans in the recombinant protein preparation.

9. The method of any one of claims 1-8, wherein the target value is one or more of:

(a) 0.1% to 20% high mannose glycans,

(b) 1% to 95% galactosylated glycans, and

(c) 70% to 100% fucosylated glycans.

10. The method of any one of claims 1-9, wherein the culture medium comprises an elevated level of ammonium.

11. The method of claim 10, wherein the elevated level of ammonium is 1 mM to 50 mM ammonium.

12. The method of claim 10, wherein the target value of the high mannose glycans is higher than a corresponding level in a preparation produced by culturing the cell in the medium not comprising the elevated level of ammonium.

13. The method of claim 12, wherein the target value is higher than the corresponding level by at least 10% of the corresponding level.

14. The method of claim 10, wherein the target value of the one or more of galactosylated glycans and fucosylated glycans is lower than a corresponding level in a preparation produced by culturing the cell in the medium not comprising the elevated level of ammonium.

15. The method of claim 14, wherein the target value is lower than the corresponding level by at least 10% of the corresponding level.

16. The method of any one of claims 1-15, wherein the culture medium further comprises one or more of glucosamine, lysine, copper, putrescine, glucose, a growth factor, a vitamin, a lipid, a peptone, and DMSO.

17. A method of producing a recombinant protein preparation, the method comprising:

(a) providing a target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans;

(b) providing a cell genetically engineered to express a recombinant protein;

(c) culturing the cell in a culture medium comprising ammonium under conditions in which the cell expresses the recombinant protein;

(d) harvesting a preparation of the recombinant protein produced by the cell; and

(e) formulating the preparation into a drug product if the preparation meets the target value of the one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans.

18. The method of claim 17, further comprising evaluating a level of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans in the recombinant protein preparation.

19. The method of claim 17 or claim 18, wherein the culture medium further comprises one or more of glucosamine, lysine, copper, putrescine, glucose, a growth factor, a vitamin, a lipid, a peptone, and DMSO.

20. A method of increasing a level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans in a recombinant protein preparation, the method comprising:

(a) providing a cell genetically engineered to express a recombinant protein;

(b) culturing the cell in a culture medium comprising an elevated level of ammonium under conditions in which the cell expresses the recombinant protein; and

(c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has an increased level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans relative to a corresponding level in a preparation of the recombinant protein produced using the medium not comprising the elevated level of ammonium.

21. The method of claim 20, further comprising measuring a level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans.

22. The method of claim 20 or claim 21, wherein the increased level of the one or more of high mannose glycans, afucosylated glycans, and G0F glycans is higher than the corresponding level by at least 10% of the corresponding level.

23. The method of any one of claims 20-22, wherein the culture medium further comprises one or more of glucosamine, lysine, copper, putrescine, glucose, a growth factor, a vitamin, a lipid, a peptone, and DMSO.

24. A method of decreasing a level of one or more of galactosylated glycans and fucosylated glycans in a recombinant protein preparation, the method comprising:

(a) providing a cell genetically engineered to express a recombinant protein;

(b) culturing the cell in a culture medium comprising an elevated level of ammonium under conditions in which the cell expresses the recombinant protein; and

(c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has a decreased level of one or more of galactosylated glycans and fucosylated glycans relative to corresponding level in a preparation of the recombinant protein produced using the medium not comprising the elevated level of ammonium.

25. The method of claim 24, wherein the fucosylated glycans comprise one or more of G1F glycans and G2F glycans.

26. The method of claim 24 or claim 25, further comprising measuring a level of one or more of galactosylated glycans and fucosylated glycans.

27. The method of any one of claims 24-26, wherein the decreased level of the one or more of galactosylated glycans and fucosylated glycans is lower than the corresponding level by at least 10% of the corresponding level.

28. The method of any one of claims 24-27, wherein the culture medium further comprises one or more of glucosamine, lysine, copper, putrescine, glucose, a growth factor, a vitamin, a lipid, a peptone, and DMSO.

29. The method of any one of claims 1-28, wherein the culture medium comprises 10 mM to 20 mM ammonium.

30. The method of any one of claims 1-29, wherein the culture medium comprises 15 mM ammonium.

31. The method of any one of claims 1-28, wherein the culturing step comprises a first stage and a second stage.

32. The method of claim 31, wherein the first stage comprises culturing the cell in the culture medium comprising a first level of ammonium, and the second stage comprises culturing the cell in the culture medium comprising a second level of ammonium.

33. The method of claim 32, wherein the second level is 1 mM to 50 mM ammonium.

34. The method of claim 32 or 33, wherein the second level is 10 mM to 20 mM ammonium.

35. The method of any one of claims 32-34, wherein the second level is 15 mM ammonium.

36. The method of any one of claims 31-35, wherein the first stage comprises culturing the cell in the first level of ammonium for 1 to 8 days.

37. The method of any one of claims 31-36, wherein the second stage comprises culturing the cell in the second level of ammonium for 1 to 12 days.

38. The method of any one of claims 1-37, wherein the cell is a CHO cell.

39. A method of producing a recombinant protein preparation having a target value of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans, the method comprising:

(a) providing a cell genetically engineered to express a recombinant protein;

(b) culturing the cell in a culture medium comprising lysine under conditions in which the cell expresses the recombinant protein; and

(c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has the target value of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans.

40. The method of claim 39, wherein the recombinant protein is a recombinant therapeutic protein.

41. The method of claim 39, wherein the recombinant protein is a recombinant therapeutic antibody.

42. The method of any one of claims 39-41, wherein the target value is a level of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans in a reference therapeutic product.

43. The method of any one of claims 39-41, wherein the target value is a level of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans in a reference therapeutic antibody product.

44. The method of any one of claims 39-41, wherein the target value is a predetermined pharmaceutical product specification.

45. The method of claim 42, wherein the reference therapeutic product is selected from the group consisting of: abatacept, abciximab, adalimumab, aflibercept, alefacept, alemtuzumab, basiliximab, bevacizumab, belatacept, certolizumab, cetuximab, daclizumab, eculizumab, efalizumab, entanercept, gemtuzumab, ibritumomab, infliximab, muromonab-CD3, natalizumab, omalizumab, palivizumab; panitumumab, ranibizumab, rilonacept, rituximab, tositumomab, and trastuzumab.

46. The method of any one of claims 39-45, further comprising evaluating a level of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans in the recombinant protein preparation.

47. The method of any one of claims 39-46, wherein the target value is one or more of:

(a) 0.1% to 20% high mannose glycans,

(b) 1% to 95% galactosylated glycans,

(c) 70% to 100% fucosylated glycans, and

(d) 0.1% to 90% sialylated glycans.

48. The method of any one of claims 39-47, wherein the culture medium comprises an elevated level of lysine.

49. The method of claim 48, wherein the elevated level of lysine is 2 g/L to 30 g/L lysine.

50. The method of claim 48 or claim 49, wherein the target value of the high mannose glycans is higher than a corresponding level in a preparation produced by culturing the cell in the medium not comprising the elevated level of lysine.

51. The method of claim 50, wherein the target value is higher than the corresponding level by at least 10% of the corresponding level.

52. The method of claim 48, wherein the target value of the one or more of galactosylated glycans, sialylated glycans, and fucosylated glycans is lower than a corresponding level in a preparation produced by culturing the cell in the medium not comprising the elevated level of lysine.

53. The method of claim 52, wherein the target value is lower than the corresponding level by at least 10% of the corresponding level.

54. The method of any one of claims 39-53, wherein the culture medium further comprises one or more of glucosamine, ammonium, copper, putrescine, glucose, a growth factor, a vitamin, a lipid, a peptone, and DMSO.

55. A method of producing a recombinant protein preparation, the method comprising:

(a) providing a target value of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans;

(b) providing a cell genetically engineered to express a recombinant protein;

(c) culturing the cell in a culture medium comprising lysine under conditions in which the cell expresses the recombinant protein;

(d) harvesting a preparation of the recombinant protein produced by the cell; and

(e) formulating the preparation into a drug product if the preparation meets the target value of the one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans.

56. The method of claim 55, further comprising evaluating a level of one or more of high mannose glycans, galactosylated glycans, fucosylated glycans, and sialylated glycans in the recombinant protein preparation.

57. The method of claim 56 or claim 57, wherein the culture medium further comprises one or more of glucosamine, ammonium, copper, putrescine, glucose, a growth factor, a vitamin, a lipid, a peptone, and DMSO.

58. A method of increasing a level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans in a recombinant protein preparation, the method comprising:

(a) providing a cell genetically engineered to express a recombinant protein;

(b) culturing the cell in a culture medium comprising an elevated level of lysine under conditions in which the cell expresses the recombinant protein; and

(c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has an increased level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans relative to a corresponding level in a preparation of the recombinant protein produced using the medium not comprising the elevated level of lysine.

59. The method of claim 58, further comprising measuring a level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans.

60. The method of claim 58 or claim 59, wherein the increased level of the one or more of high mannose glycans, afucosylated glycans, and G0F glycans is higher than the corresponding level by at least 10% of the corresponding level.

61. The method of any one of claims 58-60, wherein the culture medium further comprises one or more of glucosamine, ammonium, copper, putrescine, glucose, a growth factor, a vitamin, a lipid, a peptone, and DMSO.

62. A method of decreasing a level of one or more of galactosylated glycans, fucosylated glycans, and sialylated glycans in a recombinant protein preparation, the method comprising:

(a) providing a cell genetically engineered to express a recombinant protein;

(b) culturing the cell in a culture medium comprising an elevated level of lysine under conditions in which the cell expresses the recombinant protein; and

(c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has a decreased level of one or more of galactosylated glycans, fucosylated glycans, and sialylated glycans relative to a corresponding level in a preparation of the recombinant protein produced using the medium not comprising the elevated level of lysine.

63. The method of claim 62, wherein the fucosylated glycans comprise one or more of G1F glycans and G2F glycans.

64. The method of claim 62 or claim 63, further comprising measuring a level of one or more of galactosylated glycans, fucosylated glycans, and sialylated glycans.

65. The method of any one of claims 62-64, wherein the decreased level of one or more of galactosylated glycans, fucosylated glycans, and sialylated glycans is lower than the corresponding level by at least 10% of the corresponding level.

66. The method of any one of claims 62-65, wherein the culture medium further comprises one or more of glucosamine, ammonium, copper, putrescine, glucose, a growth factor, a vitamin, a lipid, a peptone, and DMSO.

67. The method of any one of claims 39-66, wherein the culture medium comprises 2 g/L to 10 g/L lysine.

68. The method of any one of claims 39-67, wherein the culture medium comprises 5 g/L lysine.

69. The method of any one of claims 39-68, wherein the culturing step comprises a first stage and a second stage.

70. The method of claim 69, wherein the first stage comprises culturing the cell in the culture medium comprising a first level of lysine, and the second stage comprises culturing the cell in the culture medium comprising a second level of lysine.

71. The method of claim 70, wherein the second level is 2 g/L to 30 g/L lysine.

72. The method of claim 70, wherein the second level is 2 g/L to 10 g/L lysine.

73. The method of claim 70, wherein the second level is 5 g/L lysine.

74. The method of any one of claims 70-73, wherein the first stage comprises culturing the cell in the first level of lysine for 1 to 8 days.

75. The method of any one of claims 70-74, wherein the second stage comprises culturing the cell in the second level of lysine for 1 to 12 days.

76. The method of any one of claims 39-75, wherein the cell is a CHO cell.

77. A method of producing a recombinant protein preparation having a target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans, the method comprising:

(a) providing a cell genetically engineered to express a recombinant protein;

(b) culturing the cell in a culture medium comprising a pH modifying agent under conditions in which the cell expresses the recombinant protein; and

(c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has the target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans.

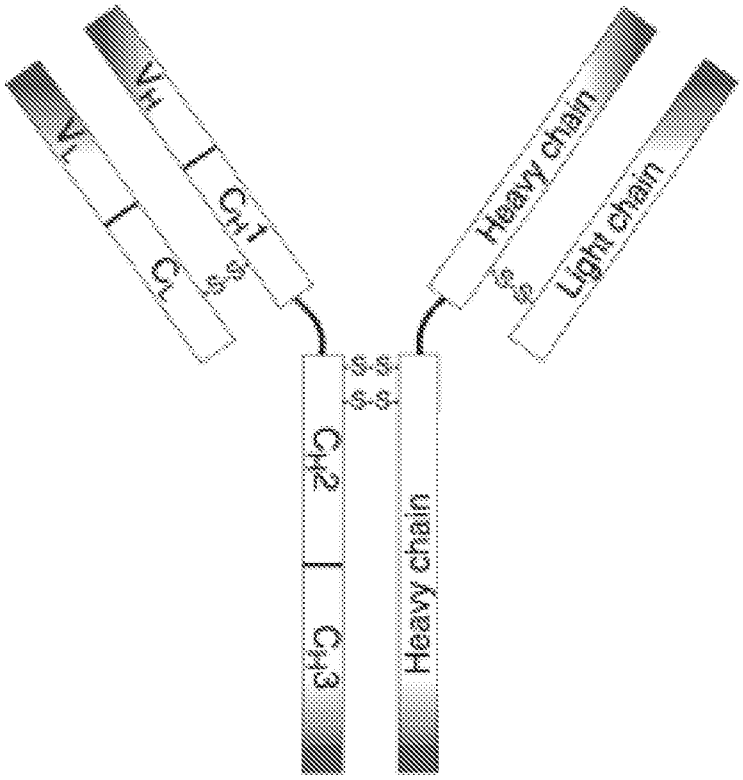
V_L
V_H
C_L
C_{H1}
S-S
Heavy chain
S
S
Light chain
S-S
S-S
C_{H2}
C_{H3}
Heavy chain

FIG. 1

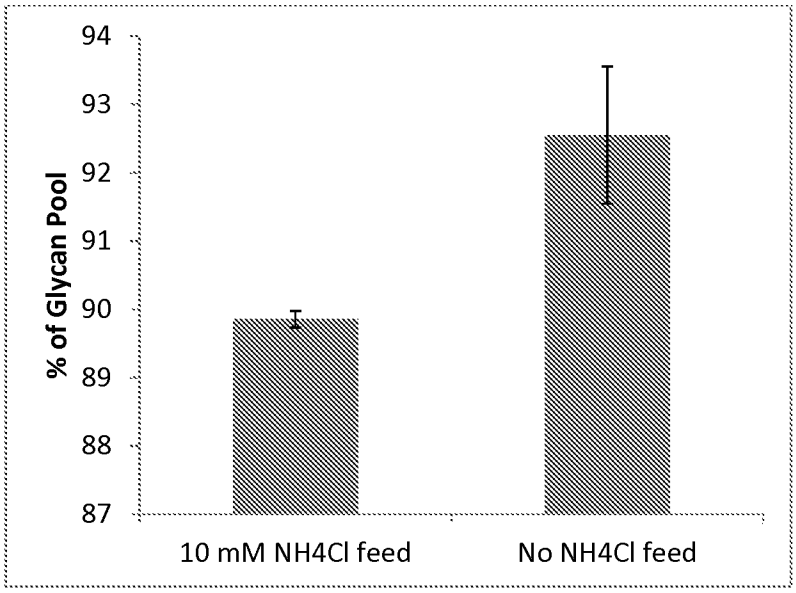
94
93
92
91
90
89
88
87
% of Glycan Pool
10 mM NH4Cl feed
No NH4Cl feed

FIG. 2A

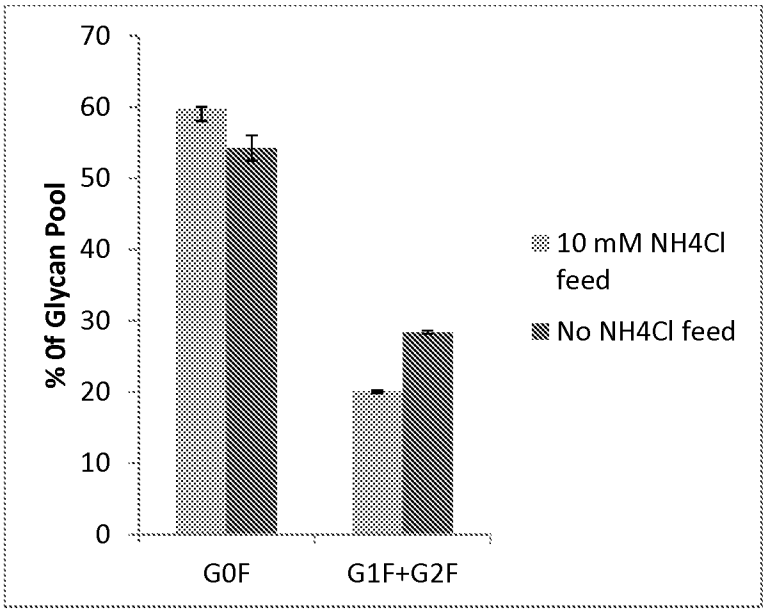
70
60
50
40
30
20
10
0
% 0f Glycan Pool
G0F
G1F+G2F
10 mM NH4Cl feed
No NH4Cl feed

FIG. 2B

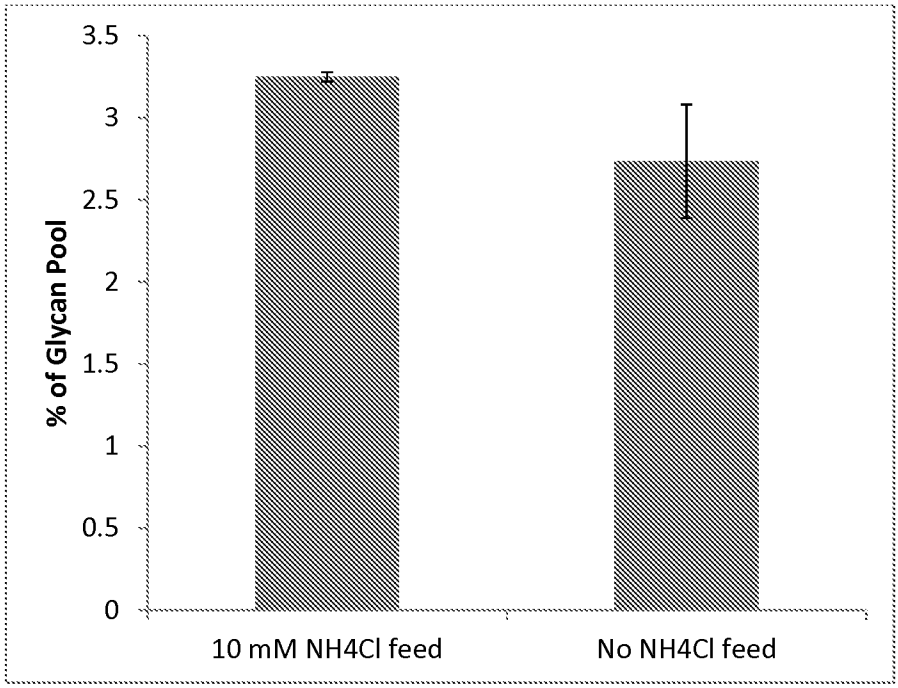

FIG. 2C

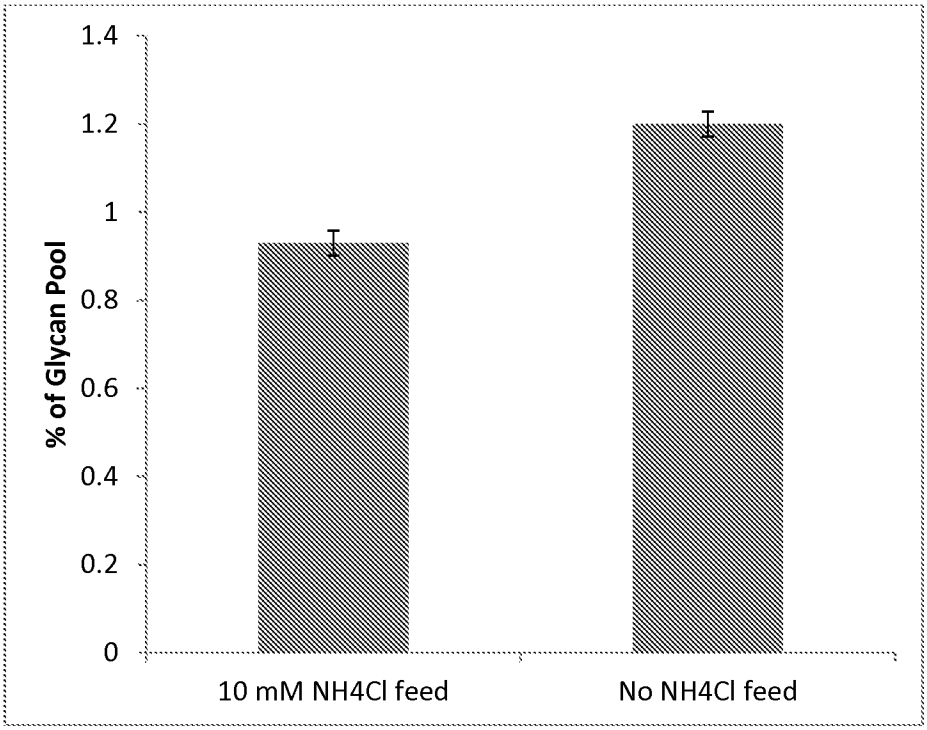
1.4
1.2
1
0.8
0.6
0.4
0.2
0
% of Glycan Pool
10 mM NH4Cl feed
No NH4Cl feed

FIG. 3

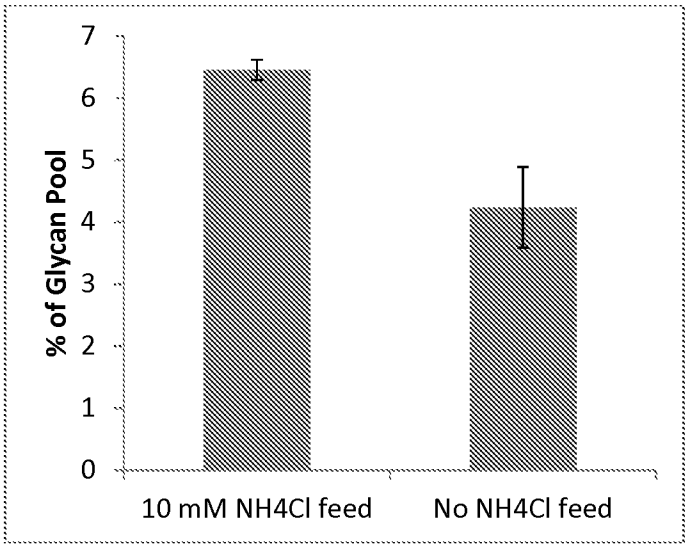
7
6
5
4
3
2
1
0
% of Glycan Pool
10 mM NH4Cl feed
No NH4Cl feed

FIG. 4A

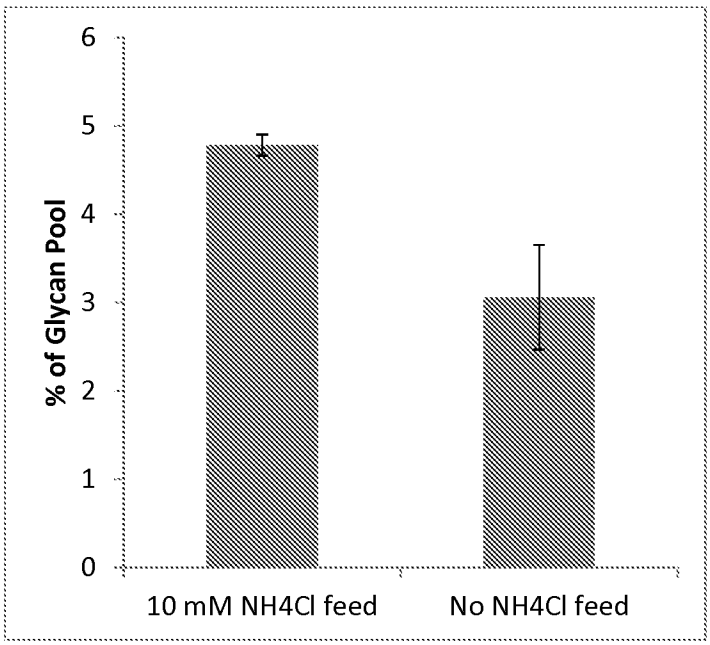
6
5
4
3
2
1
0
% of Glycan Pool
10 mM NH4Cl feed
No NH4Cl feed

FIG. 4B

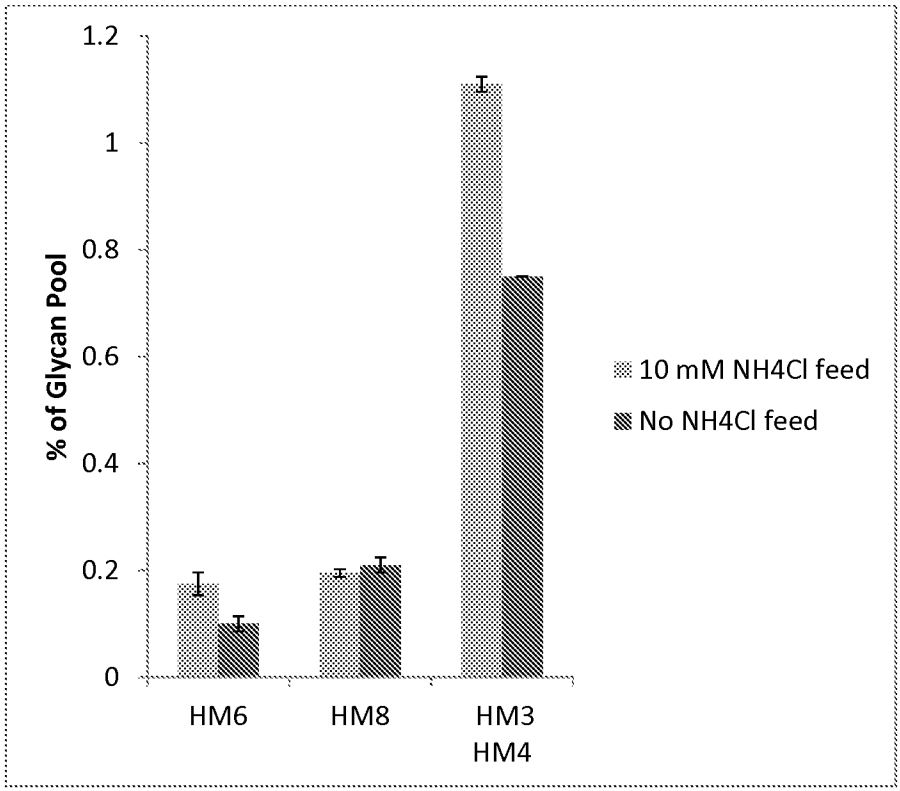
1.2
1
0.8
0.6
0.4
0.2
0
% of Glycan Pool
HM6
HM8
HM3
HM4
10 mM NH4Cl feed
No NH4Cl feed

FIG. 4C

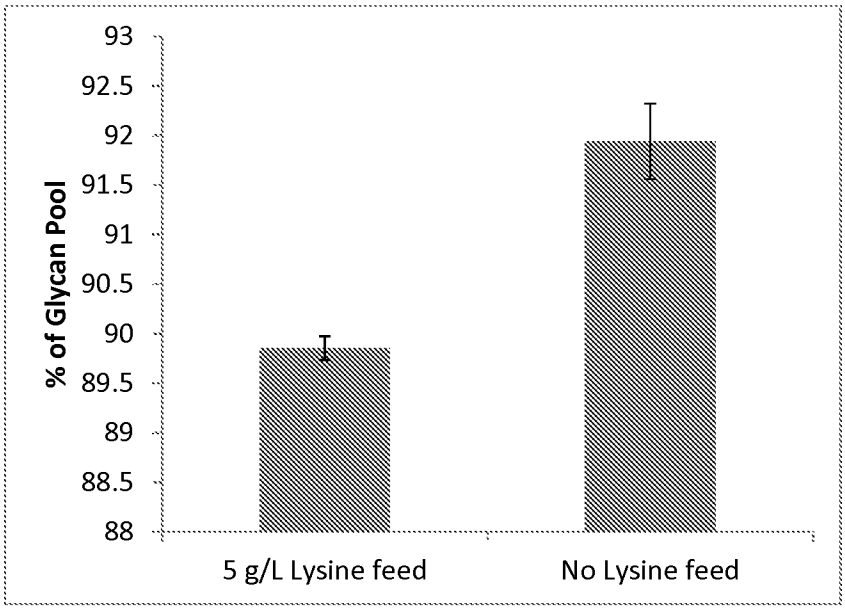
93
92.5
92
91.5
91
90.5
90
89.5
89
88.5
88
% of Glycan Pool
5 g/L Lysine feed
No Lysine feed

FIG. 5A

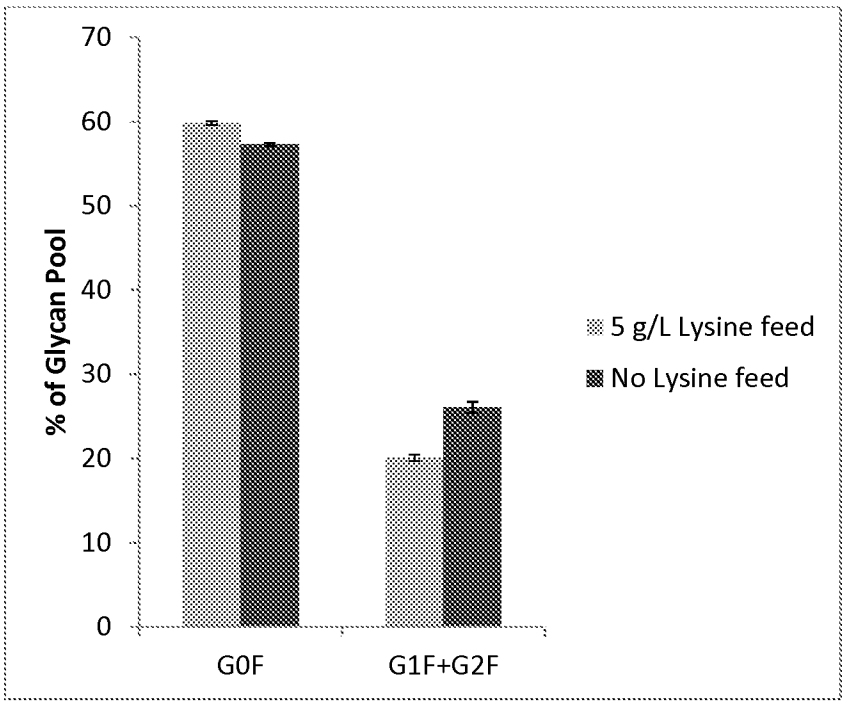
70
60
50
40
30
20
10
0
% of Glycan Pool
G0F
G1F+G2F
5 g/L Lysine feed
No Lysine feed

FIG. 5B

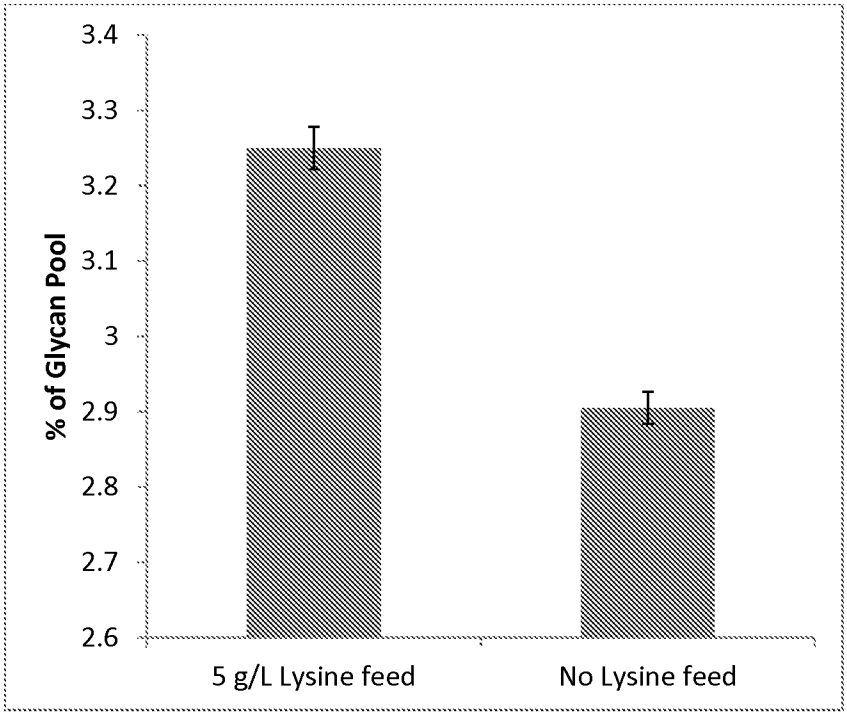
3.4
3.3
3.2
3.1
3
2.9
2.8
2.7
2.6
% of Glycan Pool
5 g/L Lysine feed
No Lysine feed

FIG. 5C

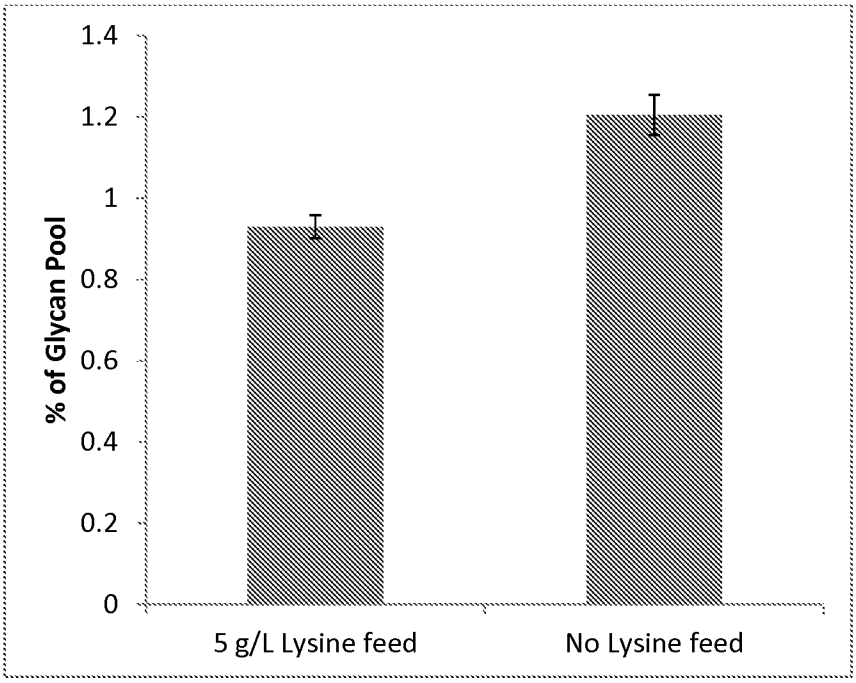
1.4
1.2
1
0.8
0.6
0.4
0.2
0
% of Glycan Pool
5 g/L Lysine feed
No Lysine feed

FIG. 6

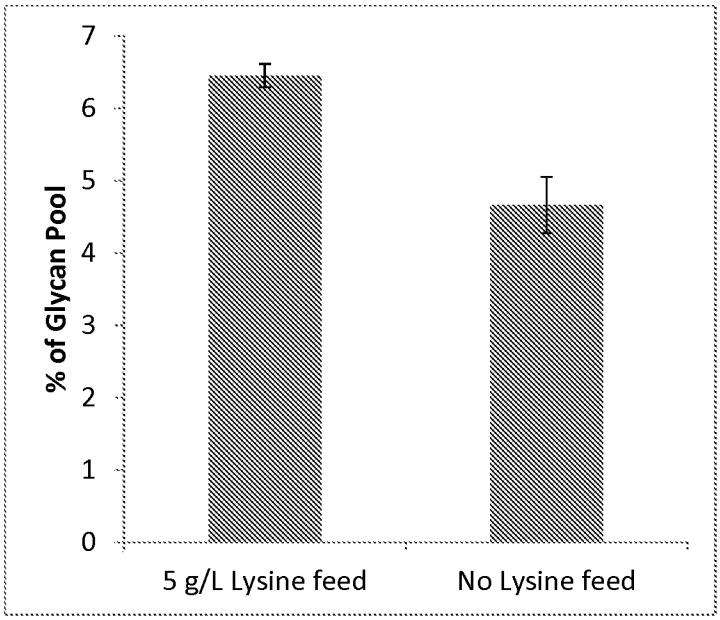
7
6
5
4
3
2
1
0
% of Glycan Pool
5 g/L Lysine feed
No Lysine feed

FIG. 7A

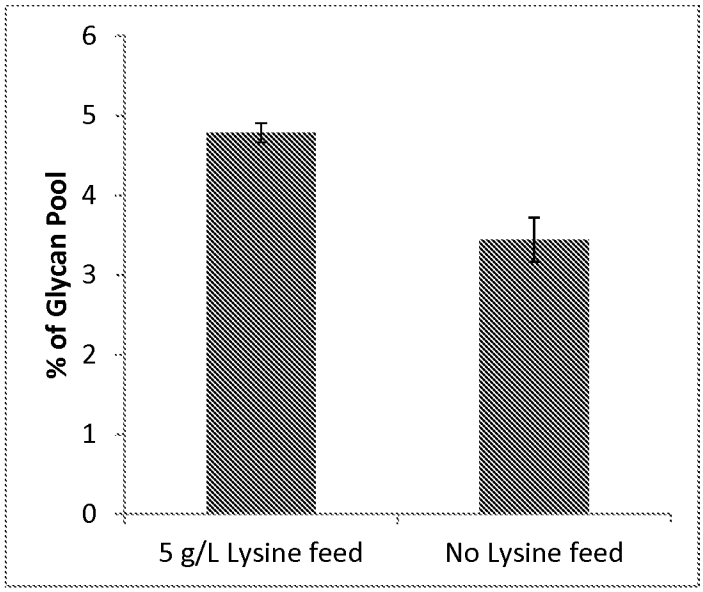
6
5
4
3
2
1
0
% of Glycan Pool
5 g/L Lysine feed
No Lysine feed

FIG. 7B

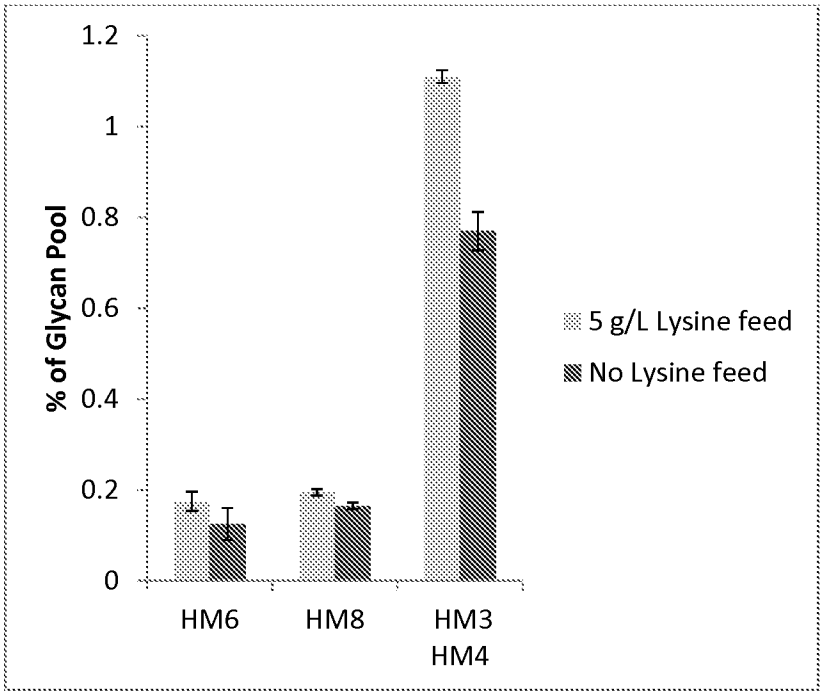
1.2
1
0.8
0.6
0.4
0.2
0
% of Glycan Pool
HM6
HM8
HM3
HM4
5 g/L Lysine feed
No Lysine feed

FIG. 7C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/23900

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12P 1/00; C12N 5/00, 5/02 (2014.01)
USPC - 435/41, 70.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C12P 1/00, 21/04, 21/06; C12N 5/00, 5/02 (2014.01)
USPC: 435/41, 70.1, 325, 69.1, 404; CPC: C12N 15/52, 9/88; C12P 1/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google Scholar; PubMed; ScienceDirect; 'high mannose,' glycoprotein, glycosylation, fucosylation, galatosylation, ammonia, ammonium

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2010/0113294 A1 (VENKATARAMAN, G et al.) May 06, 2010; abstract; paragraphs [0004], [0007], [0127], [0141], [0205], [00274], [00279], [0283], [0299], [0317], [0318]	1-3, 17-21, 24, 26/24 ----- 22/20, 22/21, 25, 26/25
Y	US 2012/0295273 A1 (WASHBURN, N et al.) November 22, 2012; paragraph [0042]	22/20, 22/21, 25, 26/25
A	US 2010/0137195 A1 (WEBER, U et al.) June 3, 2010; abstract	1-3, 17-22, 24-26

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 August 2014 (26.08.2014)	10 SEP 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/23900

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-16, 23, 27-38, 46-54, 61, 65-76
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
:***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Group I: Claims 1-3, 17-22, 24-26

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/23900

-***-Continued from Box No. III: Observations Where Unity of Invention Is Lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-3, 17-22 and 24-26 are directed toward methods of producing a recombinant protein preparation having a target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans, increasing the level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans in a recombinant protein preparation; and decreasing a level of one or more of galactosylated glycans and fucosylated glycans in a recombinant protein preparation; the methods comprising: culturing a cell in a culture medium comprising ammonium.

Group II: Claims 39-45, 55-60 and 62-64 are directed toward methods of producing a recombinant protein preparation having a target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans, increasing the level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans in a recombinant protein preparation; and decreasing a level of one or more of galactosylated glycans and fucosylated glycans in a recombinant protein preparation; the methods comprising: culturing a cell in a culture medium comprising lysine.

Group III: Claim 77 is directed toward a method of producing a recombinant protein preparation having a target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans, the method comprising culturing a cell in a culture medium comprising a pH modifying agent.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include a culture medium comprising ammonium, which are not present in Groups II or III; the special technical features of Group II including a culture medium comprising lysine, which is not present in Groups I or III; the special technical features of Group III including a culture medium comprising a pH modifying agent.

Groups I-III share the technical features including a method of producing a recombinant protein preparation having a target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans, the method comprising: (a) providing a cell genetically engineered to express a recombinant protein; (b) culturing the cell in a culture medium comprising an agent under conditions in which the cell expresses the recombinant protein; and (c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has the target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans. Groups I and II share the technical features including a method of producing a recombinant protein preparation, the method comprising: (a) providing a target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans; (b) providing a cell genetically engineered to express a recombinant protein; (c) culturing the cell in a culture medium comprising an agent under conditions in which the cell expresses the recombinant protein; (d) harvesting a preparation of the recombinant protein produced by the cell; and (e) formulating the preparation into a drug product if the preparation meets the target value of the one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans; a method of increasing a level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans in a recombinant protein preparation, the method comprising: (a) providing a cell genetically engineered to express a recombinant protein; (b) culturing the cell in a culture medium comprising an elevated level of an agent under conditions in which the cell expresses the recombinant protein; and (c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has an increased level of one or more of high mannose glycans, afucosylated glycans, and G0F glycans relative to a corresponding level in a preparation of the recombinant protein produced using the medium not comprising the elevated level of the agent; and a method of decreasing a level of one or more of galactosylated glycans and fucosylated glycans in a recombinant protein preparation, the method comprising: (a) providing a cell genetically engineered to express a recombinant protein; (b) culturing the cell in a culture medium comprising an elevated level of an agent under conditions in which the cell expresses the recombinant protein; and (c) harvesting a preparation of the recombinant protein produced by the cell, wherein the preparation has a decreased level of one or more of galactosylated glycans and fucosylated glycans relative to corresponding level in a preparation of the recombinant protein produced using the medium not comprising the elevated level of the agent.

-***-Continued on Next Supplemental Page-***-

-***-Continued from Previous Supplemental Page:

However, these shared technical features are previously disclosed by US 2010/0113294 A1 to Venkataraman, et al. (hereinafter 'Venkataraman'). Venkataraman discloses a method of producing a recombinant protein preparation (a method of producing a glycoprotein (a method of producing a recombinant protein preparation); paragraphs [0007], [0254], [0279]) having a target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans (having defined glycan properties including high mannose glycans, galactosylated glycans, and fucosylated glycans (having a target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans); paragraphs [0007], [0127]), the method comprising: (a) providing a cell genetically engineered to express a recombinant protein (providing a cell genetically engineered to express a recombinant protein; paragraphs [0254], [0279]); (b) culturing the cell in a culture medium (culturing the cell in a culture medium; paragraphs [0205], [0283], [0312]) comprising an agent (comprising an agent; paragraph [0288]; Table IV) under conditions in which the cell expresses the recombinant protein (under conditions in which the cell expresses the recombinant protein; paragraph [0141]); and (c) harvesting a preparation of the recombinant protein produced by the cell (harvesting a preparation of the recombinant protein produced by the cell; paragraphs [0317], [0318]), wherein the preparation has the target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans (wherein the preparation has the target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans; paragraphs [0127], [0141]); a method of producing a recombinant protein preparation (a method of producing a glycoprotein (a method of producing a recombinant protein preparation); paragraphs [0007], [0254], [0279]), the method comprising: (a) providing a target value of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans (providing defined glycan properties (providing a target value) of one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans; paragraphs [0007], [0127]); (b) providing a cell genetically engineered to express a recombinant protein (providing a cell genetically engineered to express a recombinant protein; paragraphs [0254], [0279]); (c) culturing the cell in a culture medium (culturing the cell in a culture medium; paragraphs [0205], [0283], [0312]) comprising an agent (comprising an agent; paragraph [0288]; Table IV) under conditions in which the cell expresses the recombinant protein (under conditions in which the cell expresses the recombinant protein; paragraph [0141]); (d) harvesting a preparation of the recombinant protein produced by the cell (harvesting a preparation of the recombinant protein produced by the cell; paragraphs [0317], [0318]); and (e) formulating the preparation into a drug product (formulating the preparation into a drug product; paragraph [0138]) if the preparation meets the target value of the one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans (if the preparation has selected glycoprotein properties, including high mannose glycans, galactosylated glycans and fucosylated glycans (if the preparation meets the target value of the one or more of high mannose glycans, galactosylated glycans, and fucosylated glycans; paragraphs [0127], [0137]); a method of increasing a level of one or more of high mannose glycans, afucosylated glycans in a recombinant protein preparation (a method of producing a protein with a modulated amount of a glycan characteristic, including high mannose glycans (a method of increasing a level of one or more of high mannose glycans in a recombinant protein preparation); paragraphs [0127], [0213], [0287]), the method comprising: (a) providing a cell genetically engineered to express a recombinant protein (providing a cell genetically engineered to express a recombinant protein; paragraphs [0254], [0279]); (b) culturing the cell in a culture medium (culturing the cell in a culture medium; paragraphs [0205], [0283], [0312]) comprising an elevated level of an agent (comprising an elevated level of an agent; paragraphs [0287], [0288]; Table IV) under conditions in which the cell expresses the recombinant protein (under conditions in which the cell expresses the recombinant protein; paragraph [0141]); and (c) harvesting a preparation of the recombinant protein produced by the cell (harvesting a preparation of the recombinant protein produced by the cell; paragraphs [0317], [0318]), wherein the preparation has an increased level (wherein the preparation has an increased level; paragraphs [0213], [0287]) of one or more of high mannose glycans (of one or more high mannose glycans; paragraph [0127]), relative to a corresponding level in a preparation of the recombinant protein produced using the medium not comprising the elevated level of the agent (according to a positive correlation of the glycan property with the media component (relative to a corresponding level in a preparation of the recombinant protein produced using the medium not comprising the elevated level of the agent; paragraph [0287]); and a method of decreasing a level of one or more of galactosylated glycans and fucosylated glycans (a method of decreasing the level of one or more of galactosylated glycans and fucosylated glycans; paragraph [0127]) in a recombinant protein preparation (in a recombinant protein preparation; paragraphs [0007], [0254]), the method comprising: (a) providing a cell genetically engineered to express a recombinant protein (providing a cell genetically engineered to express a recombinant protein; paragraphs [0254], [0279]); (b) culturing the cell in a culture medium (culturing the cell in a culture medium; paragraphs [0205], [0283], [0312]) comprising an elevated level of an agent (comprising an elevated level of an agent; paragraphs [0287], [0288]; Table IV) under conditions in which the cell expresses the recombinant protein (under conditions in which the cell expresses the recombinant protein; paragraph [0141]); and (c) harvesting a preparation of the recombinant protein produced by the cell (harvesting a preparation of the recombinant protein produced by the cell; paragraphs [0317], [0318]), wherein the preparation has a decreased level of one or more of galactosylated glycans and fucosylated glycans relative to corresponding level in a preparation of the recombinant protein produced using the medium not comprising the elevated level of the agent (wherein the preparation has a decreased level of one or more of galactosylated glycans and fucosylated glycans relative to corresponding level in a preparation of the recombinant protein produced using the medium not comprising the elevated level of the agent; paragraph [0127]; by way of example, the addition of glucosamine (X1) results in a decrease in galactosylation (Y1), a decrease in fucosylation (Y2), an increase in high mannose structures (Y3), and an increase in hybrid structures (Y4)).

Since none of the special technical features of the Groups I-III inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Venkataraman reference, unity of invention is lacking.