and *in vivo* clearance of therapeutic glycoproteins have made monitoring and control of glycosylation a critical parameter for biopharmaceutical manufacturing. The high mannose glycoform content of therapeutic proteins is a critical quality attribute that has been found to affect pharmacokinetic properties of certain therapeutic antibodies (Goetze, et al., (2011) Glycobiology 21, 949-59; Yu, et al., (2012) *MAbs 4*, 475-87). Therefore, methods for controlling the high mannose glycoform content of therapeutic proteins would be beneficial.

There is a need in the pharmaceutical industry to manipulate and control the high mannose glycoform content of recombinant therapeutic glycoproteins and methods for doing such would be useful. The invention provides a method for manipulating the high mannose glycoform content of recombinant glycoproteins by regulating ornithine metabolism in the host cells.

SUMMARY OF THE INVENTION

The invention provides in one aspect a method for decreasing high mannose glycoform content of a recombinant protein comprising culturing a host cell expressing the recombinant protein in a cell culture comprising an arginase inhibitor, wherein ornithine metabolism in the host cell is decreased when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking the arginase inhibitor, and the recombinant protein has a decreased high mannose glycoform content than when the recombinant protein is expressed in the cell culture lacking the arginase inhibitor, wherein in case the arginase inhibitor is N^G-hydroxy-L-arginine monoacetate, the concentration of N^G-hydroxy-L-arginine monoacetate is between 1 μM and 10 μM. In another aspect, the present invention refers to a method of decreasing high mannose glycoform content of a recombinant protein comprising culturing a host cell expressing the recombinant protein in a cell culture comprising spermine, wherein ornithine metabolism in the host cell is decreased when compared to the host cell expressing the recombinant protein is cultured in a cell culture with a greater concentration of spermine, and the recombinant protein has a decreased high mannose glycoform content than the recombinant protein expressed by the cell culture having the greater concentration of spermine. In still another aspect, the present invention refers to a method of increasing high mannose glycoform content of a

recombinant protein comprising culturing a host cell expressing the recombinant protein in a cell culture comprising one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase, a nitric oxide synthase inhibitor, and an arginine decarboxylase inhibitor, wherein ornithine metabolism in the host cell is decreased by increasing the accumulation of ornithine when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase, a nitric oxide synthase inhibitor and an arginine decarboxylase inhibitor, and the recombinant protein has an increased high mannose glycoform content than when the recombinant protein is expressed in the cell culture lacking one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase, a nitric oxide synthase inhibitor and an arginine decarboxylase inhibitor. In one aspect, the present invention refers to a method of producing a recombinant protein wherein the high mannose glycoform content is reduced comprising culturing a host cell which expresses the recombinant protein in a cell culture comprising an arginase inhibitor, wherein ornithine metabolism in the host cell is decreased when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking the arginase inhibitor, and the recombinant protein has a decreased high mannose glycoform content when compared to the recombinant protein in the cell culture lacking the arginase inhibitor. In another aspect, the present invention refers to a method of producing a recombinant protein wherein the high mannose glycoform content is increased comprising culturing a host cell which expresses the recombinant protein in a cell culture comprising one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase, a nitric oxide synthase inhibitor, and an arginine decarboxylase inhibitor; and the recombinant protein has an increased high mannose glycoform content when compared to the recombinant protein in the cell culture lacking one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase, a nitric oxide synthase inhibitor, and an arginine decarboxylase inhibitor. Also disclosed herein is a method for manipulating the high mannose glycoform content of a recombinant protein comprising culturing a host cell expressing the recombinant protein in a cell culture under conditions that regulate ornithine metabolism in the host cell.

In one example ornithine metabolism in the host cell is regulated by decreasing the accumulation of ornithine in the host cell. In a related example ornithine accumulation in the host cell is regulated by culturing the host cell in a cell culture medium containing an arginase inhibitor or spermine. In another related example ornithine accumulation in the host cell is regulated through the addition of an arginase

inhibitor to cell culture medium. In another related example the arginase inhibitor is BEC (S-(2-boronoethyl)-l-cysteine) or DL-a-Difluoromethylornithine. In another related example the arginase inhibitor is BEC (S-(2-boronoethyl)-l-cysteine). In another related example the arginase inhibitor is DL-a-Difluoromethylornithine. In another related example the concentration of the arginase inhibitor is at least 10 μM. In another related example the concentration of the arginase inhibitor is from 10 μM to 2 mM. In yet another related example the concentration of the arginase inhibitor is 10 μM. In yet another related example the concentration of the arginase

inhibitor is 0.5 mM. In yet another related example the concentration of the arginase inhibitor is 1 mM. In yet another related example the concentration of the arginase inhibitor is 2 mM.

In another example ornithine accumulation in the host cell is regulated by adding 35 μM or less of spermine to the cell culture medium. In a related example the concentration of spermine is 7 μM to 35 μM. In another related example the concentration of spermine is 17 μM to 35 μM. In another related example the concentration of spermine is 7 μM to 17 μM. In another related example the concentration of spermine is 35 μM. In another related example the concentration of spermine is 17 μM. In another related example the concentration of spermine is 7 μM.

In another example ornithine metabolism in the host cell is regulated by increasing the accumulation of ornithine in the host cell. In a related example ornithine accumulation in the host cell is regulated by culturing the host cell in a cell culture medium containing ornithine, arginine, an ornithine decarboxylase inhibitor, an ornithine aminotransferase, a nitric oxide synthase inhibitor or an arginine decarboxylase inhibitor. In a yet another related example ornithine accumulation in the host cell is regulated by the addition of at least 0.6 mM ornithine to the cell culture medium. In a yet another related example the concentration of ornithine is from 0.6 to 14.8 mM. In a yet another related example the concentration of ornithine is from 6 to 14.8 mM. In a yet another related example the concentration of ornithine is 0.6 mM. In a yet another related example the concentration of ornithine is 6 mM.

In a yet another related example the concentration of ornithine is 14.8 mM. In another example ornithine accumulation in the host cell is regulated by the addition of at least 8.7 mM arginine to cell culture medium. In a yet another related example the concentration of arginine is from 8.7 mM to 17.5 mM. In a yet another related example the concentration of arginine is 8.7 mM. In a yet another related example the concentration of arginine is 17.5 mM.

In another example ornithine accumulation in the host cell is regulated through the addition of an ornithine decarboxylase inhibitor, a nitric oxide synthase inhibitor, an ornithine aminotransferase inhibitor, or an arginine decarboxylase inhibitor

to the cell culture medium. In a related example ornithine accumulation in the host cell is regulated through the addition of an ornithine decarboxylase inhibitor to the cell culture medium. In yet another related example the ornithine decarboxylase inhibitor is alpha-defluoromethylornithine (DMFO). In yet another related example the ornithine decarboxylase inhibitor is piperonyl butoxide (PBO).

In another related example ornithine accumulation in the host cell is regulated through the addition of an ornithine aminotransferase inhibitor to the cell culture medium. In yet another related example the ornithine aminotransferase inhibitor is 5-fluoromethylornithine (F-FMOrn). In yet another related example the host cell is regulated through the addition of a nitric oxide synthase inhibitor to the cell culture medium. In yet another related example the nitric oxide synthase inhibitor is 2-ethyl-2-thiopseudourea or N-Nitro-L-arginine and L^G-monomethyl-L-arginine. In yet another related example the nitric oxide synthase inhibitor is N-Nitro-L-arginine and L^G-monomethyl-L-arginine.

In another related example ornithine accumulation in the host cell is regulated through the addition of an arginine decarboxylase inhibitor to the cell culture medium. In yet another related example the arginine decarboxylase inhibitor is asymmetric dimethyl-arginine (ADMA).

The disclosure provides a method of producing a recombinant protein wherein the high mannose glycoform content is reduced comprising culturing a host cell which expresses the recombinant protein in a cell culture wherein ornithine metabolism is regulated by reducing ornithine accumulation in the host cell. In a related example ornithine accumulation in the host cell is reduced by culturing the host cell in a cell culture medium containing an arginase inhibitor or spermine.

In a related example ornithine accumulation in the host cell is reduced through the addition of an arginase inhibitor to the cell culture medium. In yet another related example the arginase inhibitor is BEC (S-(2-boronoethyl)-l-cysteine) or DL-a-Difluoromethylornithine. In yet another related example the arginase inhibitor is BEC (S-(2-boronoethyl)-l-cysteine). In yet another related example the arginase inhibitor is DL-a-Difluoromethylornithine. In yet another related example the arginase inhibitor is at least 10 μM. In yet another related example the arginase

inhibitor is from 10 μM to 2 mM. In yet another related example the arginase inhibitor is from 10 μM to 20 μM. In yet another related example the arginase inhibitor is 10 μM. In yet another related example the arginase inhibitor is 0.5 mM. In yet another related example the arginase inhibitor is 1 mM. In yet another related example the arginase inhibitor is 2 mM.

In another example ornithine accumulation in the host cell is reduced by culturing the host cell in a cell culture medium containing 35 μM or less of spermine in the cell culture medium. In yet another related example the concentration of spermine is 7 μM to 35 μM. In yet another related example the concentration of spermine is 17 μM to 35 μM. In yet another related example the concentration of spermine is 0.07 mL/L to 0.17 mL/L. In yet another related example the concentration of spermine is 35 μM. In yet another related example the concentration of spermine is 17 μM. In yet another related example the concentration of spermine is 7 μM.

The disclosure provides a method of producing a recombinant protein wherein the high mannose glycoform content is increased comprising culturing a host cell which expresses the recombinant protein in a cell culture wherein ornithine metabolism is regulated by increasing ornithine accumulation in the host cell. In a related example ornithine accumulation in the host cell is increased by adding ornithine, arginine, an ornithine decarboxylase inhibitor, an ornithine aminotransferase, a nitric oxide synthase inhibitor or an arginine decarboxylase inhibitor to the cell culture medium.

In another related example ornithine accumulation in the host cell is increased by the culturing the host cell in a cell culture medium containing of at least 0.6 mM ornithine. In yet another related example the concentration of ornithine is from 0.6 to 14.8 mM. In yet another related example the concentration of ornithine is from 6 to 14.8 mM. In yet another related example the concentration of ornithine is 0.6 mM. In yet another related example the concentration of ornithine is 6 mM. In yet another related example the concentration of ornithine is 14.8 mM.

In another related example ornithine accumulation in the host cell is increased by culturing the host cell in a cell culture medium containing at least 8.7 mM arginine. In yet another related example the concentration of arginine is from 8.7 mM to 17.5

mM. In yet another related example the concentration of arginine is 8.7 mM. In yet another related example the concentration of arginine is 17.5 mM.

In another related example ornithine accumulation is increased in the host cell by culturing the host cell in a cell culture medium containing an ornithine decarboxylase inhibitor, a nitric oxide synthase inhibitor, an ornithine aminotransferase inhibitor, or an arginine decarboxylase inhibitor. In another related example ornithine accumulation in the host cell-cell is increased by culturing the host cell in a cell culture medium containing an ornithine decarboxylase inhibitor. In yet another related example the ornithine decarboxylase inhibitor is alpha-defluoromethylornithine (DMFO). In yet another related example the ornithine decarboxylase inhibitor is piperonyl butoxide (PBO).

In another related example ornithine accumulation in the host cell is increased by culturing the host cell in a cell culture medium containing an ornithine aminotransferase inhibitor. In yet another related example the ornithine aminotransferase inhibitor is 5-fluoromethylornithine (F-FMOrn).

In another related example ornithine accumulation in a host cell is increased by culturing the host cell in a cell culture medium containing a nitric oxide synthase inhibitor. In yet another related example the nitric oxide synthase inhibitor is 2-ethyl-2-thiopseudourea or N-Nitro-L-arginine and L^G-monomethyl-L-arginine.

In another example ornithine accumulation in the host cell is increased by culturing the host cell in a cell culture medium containing an arginine decarboxylase inhibitor. In yet another related example the arginine decarboxylase inhibitor is asymmetric dimethyl- arginine (ADMA).

In another example the host cell expressing the recombinant protein is cultured in a batch culture, fed-batch culture, perfusion culture, or combinations thereof. In yet another related example the culture is a perfusion culture. In yet another related example perfusion comprises continuous perfusion. In yet another related example the rate of perfusion is constant. In yet another related example the perfusion is performed at a rate of less than or equal to 1.0 working volumes per day. In yet another related example the perfusion is accomplished by alternating tangential flow.

In another example the host cell expressing the recombinant protein is cultured in a bioreactor. In yet another related example the bioreactor has a capacity of at least 500L. In yet another related example the bioreactor has a capacity of at least 500L to 2000L. In yet another related example the bioreactor has a capacity of at least 1000L to 2000L. In yet another related example the bioreactor is inoculated with at least 0.5×10^6 cells/mL.

In another example the host cell expressing the recombinant protein is cultured in a serum-free cell culture medium. In another related example the serum-free culture medium is a perfusion cell culture medium. In yet another related example the host cells are mammalian cells. In yet another related example the host cells are Chinese Hamster Ovary (CHO) cells.

In another example the recombinant protein is a glycoprotein. In another example the recombinant protein is selected from the group consisting of a human antibody, a humanized antibody, a chimeric antibody, a recombinant fusion protein, or a cytokine.

In another example the methods above further comprise a step of harvesting the recombinant protein produced by the host cell.

In another example the recombinant protein produced by the host cell is purified and formulated into a pharmaceutically acceptable formulation.

In another example a recombinant protein is provided that is produced by any of the methods above. In a related example the recombinant protein is purified. In another example the recombinant protein is formulated into a pharmaceutically acceptable formulation.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 Ornithine metabolism overview. ARG, Arginase; AZ, Antizyme; AZIN, Antizyme inhibitor; P5C, Pyrroline-5-carboxylate; ASP, Aspartate; ORNT, Ornithine transporter; GATM, Glycine amidinotransferase; NOS, Nitric oxide synthase; OAT, Ornithine aminotransferase; ODC, ornithine decarboxylase; OTC, ornithine transcarbamoylase; SMS, Spermine synthase; SRS, Spermidine synthase.

What is claimed is:

1. A method for decreasing the high mannose glycoform content of a recombinant protein comprising culturing a host cell expressing the recombinant protein in a cell culture comprising an arginase inhibitor, wherein ornithine metabolism in the host cell is decreased when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking the arginase inhibitor, and
the recombinant protein has a decreased high mannose glycoform content than when the recombinant protein is expressed in the cell culture lacking the arginase inhibitor, wherein in case the arginase inhibitor is N^G-hydroxy-L-arginine monoacetate, the concentration of N^G-hydroxy-L-arginine monoacetate is between 1 μM and 10 μM.

2. The method according to claim 1, wherein ornithine accumulation in the host cell is regulated through the addition of an arginase inhibitor to the cell culture.

3. The method according to claim 2, wherein the arginase inhibitor is selected from the group consisting of N^G-hydroxy-L-arginine monoacetate, Nω-hydroxy-nor-arginine diacetate, BEC (S-(2-boronoethyl)-l-cysteine) and DL-α-difluoromethylornithine.

4. The method according to claim 2, wherein the arginase inhibitor is BEC (S-(2-boronoethyl)-l-cysteine).

5. The method according to claim 2, wherein the arginase inhibitor is DL-a-Difluoromethylornithine.

6. The method according to claim 2, wherein the arginase inhibitor is:
(a) N^G-hydroxy-L-arginine monoacetate, having a concentration of 1 μM or 10 μM;
(b) Nω-hydroxy-nor-arginine diacetate, having a concentration of 10 μM or 20 μM;
(c) BEC (S-(2-boronoethyl)-l-cysteine), having a concentration of 1 μM, 10 μM, 20 μM or 0.5 mM; or
(d) DL-α-difluoromethylornithine, having a concentration of 1 μM, 10 μM, 20 μM, 1 mM or 2 mM.

7. The method according to claim 6, wherein concentration of the arginase inhibitor is selected from the group consisting of at least 10 μM, from 10 μM to 2 mM, 0.5 mM to 2 mM, 0.5 mM to 1 mM and 1 mM to 2 mM.

8. The method according to claim 7, wherein the arginase inhibitor is selected from the group consisting of Nω-hydroxy-nor-arginine diacetate, BEC (S-(2-boronoethyl)-l-cysteine); and DL-α-difluoromethylornithine, wherein the concentration of the arginase inhibitor is 10 μM.

9. The method according to claim 7, wherein the arginase inhibitor is BEC (S-(2-boronoethyl)-l-cysteine), wherein the concentration of the arginase inhibitor is 0.5 mM.

10. The method according to claim 7, wherein the arginase inhibitor is DL-α-difluoromethylornithine, wherein the concentration of the arginase inhibitor is 1mM.

11. The method according to claim 7, wherein the arginase inhibitor is DL-α-difluoromethylornithine, wherein the concentration of the arginase inhibitor is 2mM.

12. A method of decreasing high mannose glycoform content of a recombinant protein comprising culturing a host cell expressing the recombinant protein in a cell culture comprising spermine, wherein ornithine metabolism in the host cell is decreased when compared to the host cell expressing the recombinant protein is cultured in a cell culture with a greater concentration of spermine, and

the recombinant protein has a decreased high mannose glycoform content than the recombinant protein expressed by the cell culture having the greater concentration of spermine

13. The method according to claim 12, wherein 35 μM or less of spermine is added to the cell culture medium.

14. The method according to claim 13, wherein the concentration of spermine is 7 μM to 35 μM.

15. The method according to claim 13, wherein the concentration of spermine is 17 μM to 35 μM.

16. The method according to claim 13, wherein the concentration of spermine is 7 μM to 17 μM.

17. The method according to claim 13, wherein the concentration of spermine is 35 μM.

18. The method according to claim 13, wherein the concentration of spermine is 17 μM.

19. The method according to claim 13, wherein the concentration of spermine is 7 μM.

20. A method of increasing high mannose glycoform content of a recombinant protein comprising culturing a host cell expressing the recombinant protein in a cell culture comprising one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase, a nitric oxide synthase inhibitor, and an arginine decarboxylase inhibitor, wherein ornithine metabolism in the host cell is decreased by increasing the accumulation of ornithine when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase, a nitric oxide synthase inhibitor and an arginine decarboxylase inhibitor, and

the recombinant protein has an increased high mannose glycoform content than when the recombinant protein is expressed in the cell culture lacking one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase, a nitric oxide synthase inhibitor and an arginine decarboxylase inhibitor.

21. The method according to claim 20, wherein ornithine accumulation in the host cell is increased by the addition of at least 0.6 mM ornithine to the cell culture.

22. The method according to claim 21, wherein the concentration of ornithine is from 0.6 to 14.8 mM.

23. The method according to claim 21, wherein the concentration of ornithine is from 6 to 14.8 mM.

24. The method according to claim 21, wherein the concentration of ornithine is 0.6 mM.

25. The method according to claim 21, wherein the concentration of ornithine is 6 mM.

26. The method according to claim 21, wherein the concentration of ornithine is 14.8 mM.

27. The method according to claim 20, wherein ornithine accumulation in the host cell is regulated by the addition of at least 8.7 mM arginine to cell culture medium.

28. The method according to claim 27, wherein the concentration of arginine is from 8.7 mM to 17.5 mM.

29. The method according to claim 27, wherein the concentration of arginine is 8.7 mM.

30. The method according to claim 27, wherein the concentration of arginine is 17.5 mM.

31. A method of producing a recombinant protein wherein the high mannose glycoform content is reduced comprising culturing a host cell which expresses the recombinant protein in a cell culture comprising an arginase inhibitor, wherein ornithine metabolism in the host cell is decreased when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking the arginase inhibitor, and

the recombinant protein has a decreased high mannose glycoform content when compared to the recombinant protein in the cell culture lacking the arginase inhibitor.

32. A method of producing a recombinant protein wherein the high mannose glycoform content is increased comprising culturing a host cell which expresses the recombinant protein in a cell culture comprising one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase, a nitric oxide synthase inhibitor, and an arginine decarboxylase inhibitor, wherein ornithine metabolism in the host cell is decreased by increasing the accumulation of ornithine when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase, a nitric oxide synthase inhibitor and an arginine decarboxylase inhibitor; and

the recombinant protein has an increased high mannose glycoform content when compared to the recombinant protein expressed in the cell culture lacking the one selected

from the group consisting of ornithine, arginine, an ornithine aminotransferase, a nitric oxide synthase inhibitor, and an arginine decarboxylase inhibitor.

33. The method according to any of claims 1, 12, 20, 31, or 32, wherein the host cell expressing the recombinant protein is cultured in a batch culture, fed-batch culture, perfusion culture, or combinations thereof.

34. The method according to claim 33, wherein the culture is a perfusion culture.

35. The method according to claim 34, wherein perfusion comprises continuous perfusion.

36. The method according to claim 34, wherein the rate of perfusion is constant.

37. The method according to claim 34, wherein the perfusion is performed at a rate of less than or equal to 1.0 working volumes per day.

38. The method according to claim 34, wherein the perfusion is accomplished by alternating tangential flow.

39. The method according to any of claims 1, 12, 20, 31, or 32, wherein the host cell expressing the recombinant protein is cultured in a bioreactor.

40. The method according to claim 39, wherein the bioreactor has a capacity of at least 500L.

41. The method according to claim 39, wherein the bioreactor has a capacity of at least 500L to 2000L.

42. The method according to claim 39, wherein the bioreactor has a capacity of at least 1000L to 2000L.

43. The method according to claim 39, wherein the bioreactor is inoculated with at least 0.5 x 10^6 cells/mL.

44. The method according to any of claims 1, 12, 20, 31, or 32, wherein the host cell expressing the recombinant protein is cultured in a serum-free cell culture medium.

45. The method according to any of claim 44, wherein the serum-free culture medium is a perfusion cell culture medium.

46. The method according to any of claims 1, 12, 20, 31, or 32, wherein the host cells are mammalian cells.

47. The method according to any of claims 1, 12, 20, 31, or 32, wherein the host cells are Chinese Hamster Ovary (CHO) cells.

48. The method according to any of claims 1, 12, 20, 31, or 32, wherein the recombinant protein is a glycoprotein.

49. The method according to any of claims 1, 12, 20, 31, or 32, wherein the recombinant protein is selected from the group consisting of a human antibody, a humanized antibody, a chimeric antibody, a recombinant fusion protein, or a cytokine.

50. The method according to any of claims 1, 12, 20, 31, or 32, further comprising a step of harvesting the recombinant protein produced by the host cell.

51. The method according to any of claims 1, 12, 20, 31, or 32, wherein the recombinant protein produced by the host cell is purified and formulated into a pharmaceutically acceptable formulation.