identify culture conditions and production methods that allow for control over the development of such heterogeneity. Control of protein heterogeneity is particularly advantageous in the context of cell culture processes used for commercially produced recombinant bio-therapeutics as such heterogeneity has the potential to impact therapeutic utility. The instant invention addresses this need by providing compositions and processes to control protein heterogeneity.

3. SUMMARY OF THE INVENTION

The present invention is directed to compositions and methods that control (modulate or limit) protein heterogeneity arising in a population of proteins, e.g., in the context of recombinant protein production.

In one aspect, there is provided a process for producing a recombinantly-expressed immunoglobulin comprising a heavy chain variable region comprising the sequence of SEQ ID NO: 2 and a light chain variable region comprising the sequence of SEQ ID NO: 7, comprising

culturing a mammalian cell which recombinantly expresses the immunoglobulin in a cell culture media comprising a yeast hydrolysate and/or a plant hydrolysate, thereby producing the recombinantly-expressed immunoglobulin,

wherein the level of agalactosyl fucosylated biantennary oligosaccharides (sum of NGA2F and NGA2F-GlcNac) present on the produced immunoglobulin is decreased as compared to the level of agalactosyl fucosylated biantennary oligosaccharides (sum of NGA2F and NGA2F-GlcNac) of immunoglobulin produced in cell culture media which does not comprise said yeast hydrolysate, and/or said plant hydrolysate; and/or wherein the level of galactose containing fucosylated biantennary oligosaccharides (sum of NA1F and NA2F) present on the produced immunoglobulin is increased as compared to the level of galactose containing fucosylated biantennary oligosaccharides (sum of NA1F and NA2F) of immunoglobulin produced in cell culture media which does not comprise said yeast hydrolysate and/or said plant hydrolysate; and

wherein the level of agalactosyl fucosylated biantennary oligosaccharides (sum of NGA2F and NGA2F-GlcNAc) present on the produced immunoglobulin is

66%-69%; and/or wherein the level of fucosylated biantennary oligosaccharides (sum of NA1F and NA2F) present on the produced immunoglobulin is 29%-31%.

In one aspect, there is provided a process for producing a recombinantly-expressed immunoglobulin comprising a heavy chain variable region comprising the sequence of SEQ ID NO: 2 and a light chain variable region comprising the sequence of SEQ ID NO: 7, comprising

culturing a mammalian cell which expresses the immunoglobulin during a production stage in a cell culture media comprising at least 11 g/L of a yeast hydrolysate and/or at least 7 g/L of a plant hydrolysate, thereby producing the recombinantly-expressed immunoglobulin, and

assessing the oligosaccharide distribution of the produced immunoglobulin,

wherein the level of agalactosyl fucosylated biantennary oligosaccharides (sum of NGA2F and NGA2F-GlcNac) present on the produced immunoglobulin is decreased as compared to the level of agalactosyl fucosylated biantennary oligosaccharides (sum of NGA2F and NGA2F-GlcNac) of the immunoglobulin produced in a cell culture media which does not comprise said yeast hydrolysate and/or said plant hydrolysate during the production stage; and/or

wherein the level of galactose containing fucosylated biantennary oligosaccharides (sum of NA1F and NA2F) present on the produced immunoglobulin is increased as compared to the level of galactose containing fucosylated biantennary oligosaccharides (sum of NA1F and NA2F) of the immunoglobulin produced in a cell culture media which does not comprise said yeast hydrolysate and/or said plant hydrolysate during the production stage.

In certain embodiments, the heterogeneity corresponds to the glycosylation state of individual members of a population of proteins. In certain embodiments, control is exerted over the type of glycosylation substitutions present on individual members of a population of proteins. In certain embodiments, control is exerted over the extent of glycosylation substitutions present on individual members of a population of proteins. In certain embodiments, control is exerted over both the type and extent of glycosylation substitutions present on individual members of a

population of proteins. In certain embodiments, such control results in a decrease in the amount of NGA2F+NGA2F-GlcNac oligosaccharides and/or an increase in the amount of NA1F+NA2F oligosaccharides linked to the protein of interest. In certain embodiments, such control results in an increase in the amount of NGA2F+NGA2F-GlcNac oligosaccharides and/or a decrease in the amount of NA1F+NA2F oligosaccharides linked to the protein of interest.

In certain embodiments, control over protein glycosylation heterogeneity is exerted by employing specific hydrolysates during production of the protein of interest, for example, but not by way of limitation, in adaptation cultures performed in media supplemented with hydrolysates. In certain embodiments, control over protein glycosylation heterogeneity is exerted by maintaining certain yeastolate to phytone ratios during production of the protein of interest. In certain embodiments, control over protein glycosylation heterogeneity is exerted by the addition of asparagine during the production of the protein of interest. In certain embodiments

What is claimed is:

1. A method for controlling the oligosaccharide distribution of a recombinantly-expressed protein comprising supplementing a cell culture media used in the recombinant expression of said protein with a yeast hydrolysate supplement and/or a plant hydrolysate supplement.

2. The method of claim 1 wherein the recombinantly-expressed protein is an antibody.

3. The method of claim 2 wherein the antibody is an anti-TNFα antibody.

4. The method of claim 3 wherein the anti-TNFα antibody is adalimumab.

5. The method of claim 1 wherein the yeast hydrolysate supplement is selected from the group consisting of: Bacto TC Yeastolate, HyPep Yeast Extract, and UF Yeast Hydrolysate.

6. The method of claim 1 wherein the plant hydrolysate supplement is a soy hydrolysate or a wheat, rice, cotton seed, pead, corn or potato hydrolysate.

7. The method of claim 6 wherein plant hydrolysate supplement is selected from the group consisting of: BBL Phytone Peptone; HyPep 1510; SE50 MAF-UF; UF Soy Hydrolysate; Wheat Peptone E1; HyPep 4601; and Proyield WGE80M Wheat.

8. The method of claim 1 wherein the cell culture media is supplemented with a sufficient amount of the yeast hydrolysate supplement to achieve a yeast hydrolysate concentration selected from about 2 g/L to about 11 g/L.

9. The method of claim 8 wherein the cell culture media is supplemented with a sufficient amount of the yeast hydrolysate supplement to achieve a yeast hydrolysate concentration selected from the following: 2 g/L, 5 g/L, and 11 g/L.

10. The method of claim 1 wherein the cell culture media is supplemented with a sufficient amount of the plant hydrolysate supplement to achieve a plant hydrolysate concentration selected from about 2 g/L to about 15 g/L.

11. The method of claim 10 wherein the production media is supplemented with a sufficient amount of the plant hydrolysate supplement to achieve a plant hydrolysate concentration selected from the following: 2 g/L, 4 g/L, 7 g/L, 10 g/L, and 15 g/L.

12. The method of claim 1 wherein the cell culture media is supplemented with a sufficient amount of the yeast hydrolysate supplement and the plant hydrolysate supplement to achieve the yeast hydrolysate to the plant hydrolysate ratio of about 0.25 to about 1.55.

13. The method of claim 12 wherein the cell culture media is supplemented with a sufficient amount of the yeast hydrolysate supplement and plant hydrolysate supplement to achieve a yeast hydrolysate and plant hydrolysate concentration selected from the following presented as yeast hydrolysate (g/L)/ plant hydrolysate (g/L): 0/2; 0/4; 0/7; 2/0; 5/0; 11/0; 4/2.58; and 10.7/6.92.

14. A method for controlling the oligosaccharide distribution of a recombinantly-expressed protein comprising modulating the asparagine concentration of the cell culture media used in the recombinant expression of said protein.

15. The method of claim 14 wherein the recombinantly-expressed protein is an antibody.

16. The method of claim 15 wherein the antibody is an anti-TNFα antibody.

17. The method of claim 16 wherein the anti-TNFα antibody is adalimumab.

18. The method of claim 14 wherein the total concentration of asparagine in the cell culture media is from about 0 mM to about 26 mM.

What is claimed is:

1. A process for producing a recombinantly-expressed immunoglobulin comprising a heavy chain variable region comprising the sequence of SEQ ID NO: 2 and a light chain variable region comprising the sequence of SEQ ID NO: 7, comprising

culturing a mammalian cell which recombinantly expresses the immunoglobulin in a cell culture media comprising a yeast hydrolysate and/or a plant hydrolysate, thereby producing the recombinantly-expressed immunoglobulin,

wherein the level of agalactosyl fucosylated biantennary oligosaccharides (sum of NGA2F and NGA2F-GlcNac) present on the produced immunoglobulin is decreased as compared to the level of agalactosyl fucosylated biantennary oligosaccharides (sum of NGA2F and NGA2F-GlcNac) of immunoglobulin produced in cell culture media which does not comprise said yeast hydrolysate, and/or said plant hydrolysate; and/or wherein the level of galactose containing fucosylated biantennary oligosaccharides (sum of NA1F and NA2F) present on the produced immunoglobulin is increased as compared to the level of galactose containing fucosylated biantennary oligosaccharides (sum of NA1F and NA2F) of immunoglobulin produced in cell culture media which does not comprise said yeast hydrolysate and/or said plant hydrolysate; and

wherein the level of agalactosyl fucosylated biantennary oligosaccharides (sum of NGA2F and NGA2F-GlcNAc) present on the produced immunoglobulin is 66%-69%; and/or wherein the level of fucosylated biantennary oligosaccharides (sum of NA1F and NA2F) present on the produced immunoglobulin is 29%-31%.

2. The process of claim 1, wherein the immunoglobulin is adalimumab.

3. The method of claim 2, wherein the method produces at least 2.5 g/L of adalimumab.

4. The process of claim 1, wherein the cell which expresses the immunoglobulin is a CHO cell.

5. The process of claim 1, wherein the cell culture media comprises a yeast hydrolysate and/or plant hydrolysate during a production stage.

6. The process of claim 5, wherein the production stage initiates at an initial viable cell density of approximately 0.5×10^{6}cells/mL.

7. The process of claim 1, wherein the cell culture media is a chemically defined cell culture media.

8. The process of claim 1, wherein the yeast hydrolysate is selected from the group consisting of Bacto TC Yeastolate, HyPep Yeast Extract and UF Yeast Hydrolysate; and/or wherein the plant hydrolysate is selected from the group consisting of a soy hydrolysate, a wheat hydrolysate, a rice hydrolysate, a cotton seed hydrolysate, a pea hydrolysate, a corn hydrolysate, a potato hydrolysate, BBL Phytone Peptone, HyPep 1510, SE50 MAF-UF, UF Soy Hydrolysate, Wheat Peptone E1, HyPep 4601 and Proyield WGE80M Wheat.

9. The process of claim 1, wherein the concentration of yeast hydrolysate is 2 g/L to 11 g/L.

10. The process of claim 1, wherein the concentration of plant hydrolysate is 2 g/L to 15 g/L.

11. The method of claim 10, wherein the concentration of plant hydrolysate is 7 g/L to 15 g/L.

12. The process of claim 1, wherein the level of agalactosyl fucosylated biantennary oligosaccharides (sum of NGA2F and NGA2F-GlcNAc) present on the produced immunoglobulin is 66%-69%.

13. The process of claim 1, wherein the level of fucosylated biantennary oligosaccharides (sum of NA1F and NA2F) present on the produced immunoglobulin is 29%-31%.

14. The process of claim 1, further comprising collecting and isolating the recombinantly-expressed immunoglobulin.

15. The process of claim 1, wherein the process is a fed batch process.

16. A process for producing a recombinantly-expressed immunoglobulin comprising a heavy

chain variable region comprising the sequence of SEQ ID NO: 2 and a light chain variable region comprising the sequence of SEQ ID NO: 7, comprising

culturing a mammalian cell which expresses the immunoglobulin during a production stage in a cell culture media comprising at least 11 g/L of a yeast hydrolysate and/or at least 7 g/L of a plant hydrolysate, thereby producing the recombinantly-expressed immunoglobulin, and

assessing the oligosaccharide distribution of the produced immunoglobulin,

wherein the level of agalactosyl fucosylated biantennary oligosaccharides (sum of NGA2F and NGA2F-GlcNac) present on the produced immunoglobulin is decreased as compared to the level of agalactosyl fucosylated biantennary oligosaccharides (sum of NGA2F and NGA2F-GlcNac) of the immunoglobulin produced in a cell culture media which does not comprise said yeast hydrolysate and/or said plant hydrolysate during the production stage; and/or

wherein the level of galactose containing fucosylated biantennary oligosaccharides (sum of NA1F and NA2F) present on the produced immunoglobulin is increased as compared to the level of galactose containing fucosylated biantennary oligosaccharides (sum of NA1F and NA2F) of the immunoglobulin produced in a cell culture media which does not comprise said yeast hydrolysate and/or said plant hydrolysate during the production stage.

17. The process of claim 16, further comprising collecting and isolating the recombinantly-expressed immunoglobulin.

18. The process of claim 16, wherein the process is a fed batch process.

19. The process of claim 16, wherein the immunoglobulin is adalimumab.

20. The method of claim 19, wherein the method produces at least 2.5 g/L of adalimumab.

21. The process of claim 16, wherein the production stage initiates at an initial viable cell density of approximately 0.5×10^6 cells/mL.

22. The process of claim 16, wherein the cell which expresses the immunoglobulin is a CHO cell.

23. The process of claim 16, wherein the cell culture media is a chemically defined cell culture media.

24. The process of claim 16, wherein the yeast hydrolysate is selected from the group consisting of Bacto TC Yeastolate, HyPep Yeast Extract and UF Yeast Hydrolysate; and/or wherein the plant hydrolysate is selected from the group consisting of a soy hydrolysate, a wheat hydrolysate, a rice hydrolysate, a cotton seed hydrolysate, a pea hydrolysate, a corn hydrolysate, a potato hydrolysate, BBL Phytone Peptone, HyPep 1510, SE50 MAF-UF, UF Soy Hydrolysate, Wheat Peptone E1, HyPep 4601 and Proyield WGE80M Wheat.

25. The process of claim 16, wherein the concentration of plant hydrolysate is 7 g/L-15 g/L.

26. The process of claim 16, wherein the concentration of plant hydrolysate is 10 g/L-15 g/L.

27. The process of claim 16, wherein the level of agalactosyl fucosylated biantennary oligosaccharides (sum of NGA2F and NGA2F-GlcNAc) present on the produced immunoglobulin is 64%-88%, 70%-88% or 75%-85%.

28. The process of claim 16, wherein the level of fucosylated biantennary oligosaccharides (sum of NA1F and NA2F) present on the produced immunoglobulin is 1%-30%, 2%-25%, 5%-20%, 5%-15%, 10%-20% or 27%-31%.

29. The process of claim 16, wherein the level of agalactosyl fucosylated biantennary oligosaccharides (sum of NGA2F and NGA2F-GlcNAc) present on the recombinantly-expressed immunoglobulin is 66%-69%.

30. The process of claim 16, wherein the level of fucosylated biantennary oligosaccharides (sum of NA1F and NA2F) present on the recombinantly-expressed immunoglobulin is 29%-31%.