Additionally, the present methods are not limited to production of multi-protein complexes but may also be readily adapted for use with ribonucleoprotein (RNP) complexes including telomerase, hnRNPs, Ribosomes, snRNPs, signal recognition particles, prokaryotic and eukaryotic RNase P complexes, and any other complexes that contain multiple distinct protein and / or RNA subunits. The host cell that expresses the multi-subunit complex may be produced by methods known in the art. For example, a panel of diploid or tetraploid yeast cells containing differing combinations of gene copy numbers may be generated by mating cells containing varying numbers of copies of the individual subunit genes (which numbers of copies preferably are known in advance of mating).

Definitions

[00181] It is to be understood that this invention is not limited to the particular methodology, protocols, cell lines, animal species or genera, and reagents described, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[00182] As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the protein" includes reference to one or more proteins and equivalents thereof known to those skilled in the art, and so forth. All technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs unless clearly indicated otherwise.

[00183] *Bolus addition*: In the present disclosure, "bolus addition" generally refers to rapid change in concentration of a substance (such as ethanol) in contact with cultured cells (for example, in a culture medium). For example, the substance may be added to the cultured cells in a single addition, a succession of more than one addition, and/or infused over a period of time (e.g., over about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 90, or 120 minutes). The substance may also be added by replacing the culture medium in part or in full, for example by concentrating the cells (using centrifugation, filtration, settling, or other methods), removing part or all of the medium, and adding the substance, or by adding the cells to a medium containing the

[00943] The above description of various illustrated embodiments of the invention is not intended to be exhaustive or to limit the invention to the precise form disclosed. While specific embodiments of, and examples for, the invention are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the invention, as those skilled in the relevant art will recognize. The teachings provided herein of the invention can be applied to other purposes, other than the examples described above.

[00944] The invention may be practiced in ways other than those particularly described in the foregoing description and examples. Numerous modifications and variations of the invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

[00945] These and other changes can be made to the invention in light of the above detailed description. In general, in the following claims, the terms used should not be construed to limit the invention to the specific embodiments disclosed in the specification and the claims. Accordingly, the invention is not limited by the disclosure, but instead the scope of the invention is to be determined entirely by the following claims.

[00946] Certain teachings related to methods for obtaining a clonal population of antigen-specific B cells were disclosed in U.S. Provisional patent application no. 60/801,412, filed May 19, 2006, the disclosure of which is herein incorporated by reference in its entirety.

[00947] Certain teachings related to humanization of rabbit-derived monoclonal antibodies and preferred sequence modifications to maintain antigen binding affinity were disclosed in International Application No. PCT/US2008/064421, corresponding to International Publication No. WO/2008/144757, entitled "Novel Rabbit Antibody Humanization Methods and Humanized Rabbit Antibodies", filed May 21, 2008, the disclosure of which is herein incorporated by reference in its entirety.

[00948] Certain teachings related to producing antibodies or fragments thereof using mating competent yeast and corresponding methods were disclosed in U.S. Patent application no.

11/429,053, filed May 8, 2006, (U.S. Patent Application Publication No. US2006/0270045), the disclosure of which is herein incorporated by reference in its entirety.

[00949] The entire disclosure of each document cited herein (including patents, patent applications, journal articles, abstracts, manuals, books, or other disclosures), including each document cited in the Background, Summary, Detailed Description, and Examples, is hereby incorporated by reference herein in its entirety.

Claims

1. A method of producing a multi-subunit complex, comprising:

(a) providing a culture comprising a eukaryotic cells comprising genes that provide for the expression of the subunits of said multi-subunit complex;

(b) adding a bolus of ethanol to said culture; and

(c) culturing said culture to produce said multi-subunit complex.

2. The method of **claim 1**, wherein the ethanol bolus enhances the formation of stable disulfide bonds relative to the same method effected in the absence of the bolus of ethanol.

3. The method of **claim 1**, wherein said multi-subunit complex contains one or more polypeptides comprising at least one disulfide bond.

4. The method of **claim 1**, wherein said multi-subunit complex comprises an antibody.

5. The method of any one of **claims 1 to 4**, which decreases the relative abundance of one or more product-associated variants relative to the same method effected in the absence of the bolus of ethanol.

6. The method of any one of **claims 1 to 4**, which decreases the relative abundance of product-associated variants having a higher or lower apparent molecular weight than said desired multi-subunit complex as detected by size exclusion chromatography or gel electrophoresis relative to the same method effected in the absence of the bolus of ethanol.

7. The method of any one of **claims 1 to 4**, which decreases the relative abundance of complexes having aberrant stoichiometry relative to the same method effected in the absence of the bolus of ethanol.

8. The method of any one of **claims 1 to 4**, which decreases the relative abundance of complexes having aberrant disulfide bonds relative to the same method effected in the absence of the bolus of ethanol.

9. The method of any one of **claims 1 to 4**, which decreases the relative abundance of complexes having reduced cysteines relative to the same method effected in the absence of the bolus of ethanol.

10. The method of any one of **claims 1 to 4**, which decreases the relative abundance of complexes having aberrant glycosylation relative to the same method effected in the absence of the bolus of ethanol.

11. The method of **claim 4**, which modulates the formation or stability of inter-heavy chain disulfide bonds.

12. The method of **claim 4 or 11**, which modulates the formation or stability of disulfide bonds linking the light and heavy chains.

13. The method of any one of **claims 4 or 11 to 12**, which decreases the relative abundance of one or more product-associated variants relative to the same method effected in the absence of the bolus of ethanol.

14. The method of **claim 13**, wherein said product-associated variants comprise one or more of the H1L1, H2L1, and H4L4 product-associate variants.

15. The method of any one of **claims 4 or 11 to 14**, which increases the purity of said antibody relative to said method effected in the absence of said bolus of ethanol.

16. The method of any one of the **foregoing claims**, wherein step (b) is effected prior to step (c).

17. The method of any one of **claims 1 to 15**, wherein step (b) is effected subsequent to step (c).

18. The method of any one of **claims 1 to 15**, wherein step (b) is effected concurrently with step (c).

19. The method of any one of the **foregoing claims**, wherein step (b) results in a concentration of ethanol in said culture of between about 0.01% and about 4% (w/v).

20. The method of any one of the **foregoing claims**, wherein step (b) results in a concentration of ethanol in said culture of between about 0.01% and about 4%, between about 0.02% and about 3.75%, between about 0.04% and about 3.5%, between about 0.08% and about 3.25%, between about 0.1% and about 3%, between about 0.2% and about 2.75%, between about 0.3% and about 2.5%, between about 0.4% and about 2.25%, between about 0.5% and about 1.5%, between about 0.5% and about 2%, between about 0.6% and about 1.75%, between about 0.7% and about 1.5%, or between about 0.8% and about 1.25%.

21. The method of any one of the **foregoing claims**, wherein step (b) results in a concentration of ethanol in said culture that is at least about 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.2%, 0.3%, 0.4%, 0.6%, 0.6%, 0.7%, 0.8% or 0.9% (w/v).

22. The method of any one of the **foregoing claims**, wherein step (b) results in a concentration of ethanol in said culture that is at most about 4%, 3.5%, 3%, 2.5%, 2%, 1.8%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.35%, 0.3%, 0.25%, 0.2%, or 0.15% (w/v).

23. The method of any one of the **foregoing claims**, wherein step (b) comprises adding ethanol to said culture, adding a carrier comprising ethanol to said culture, adding said cells to a medium or carrier comprising ethanol, or replacing part of the culture medium.

24. The method of any one of the **foregoing claims**, wherein said bolus of ethanol is added to the culture medium over a period of time between 1 and 20 minutes.

25. The method of any one of the **foregoing claims**, wherein step (c) comprises providing oxygen to said cells.

26. The method of **claim 25**, wherein said providing oxygen comprises agitating said culture.

27. The method of **claim 25 or 26**, wherein said providing oxygen comprises contacting said culture with a gas mixture comprising oxygen.

28. The method of any one of the **foregoing claims**, wherein step (c) comprises adding a feed comprising a carbon source to said culture.

29. The method of **claim 28**, wherein said feed comprises at least one fermentable carbon source.

30. The method of **claim 28**, wherein said feed comprises one or more of glucose, ethanol, citrate, sorbitol, xylose, trehalose, arabinose, galactose, fructose, melibiose, lactose, maltose, rhamnose, ribose, mannose, mannitol, and raffinose.

31. The method of any one of the **foregoing claims**, further comprising maintaining the concentration of ethanol between an upper set point and a lower set point during step (c).

32. The method of **claim 31**, wherein said lower set point is about 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.2%, 0.3%, 0.4%, 0.6%, 0.6%, 0.7%, 0.8% or 0.9% (w/v).

33. The method of **claim 31 or 32**, wherein said upper set point is about 4%, 3.5%, 3%, 2.5%, 2%, 1.8%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.35%, 0.3%, 0.25%, 0.2%, or 0.15% (w/v).

34. The method of **claim 31 or 32**, wherein said upper set point is at most about 1.5%, 1.4%, 1.3, 1.2%, or 1.1% (w/v).

35. The method of any one of **claims 1 to 30**, further comprising maintaining the concentration of ethanol at a set point during step (c).

36. The method of **claim 35**, wherein said set point is about 0.1%, 0.2%, 0. 3%, 0. 4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 01.%, 01.1%, 01.2%, 01.3%, 01.4%, or 01.5% (w/v).

37. The method of any one of **claims 1 to 30**, wherein step (c) comprises maintaining the concentration of ethanol in said culture between about 0.01% and about 4%, between about 0.02% and about 3.75%, between about 0.04% and about 3.5%, between about 0.08% and about 3.25%, between about 0.1% and about 3%, between about 0.2% and about 2.75%, between about 0.3% and about 2.5%, between about 0.4% and about 2.25%, between about 0.5% and about 2%, between about 0.6% and about 1.75%, between about 0.7% and about 1.5%, or between about 0.8% and about 1.25%.

38. The method of any one of claims 21 to 27, wherein the concentration of ethanol in said culture is maintained by controlling production of ethanol by said cells or by addition of ethanol to said culture.

39. The method of claim 28, wherein the step of controlling production of ethanol comprises controlling one or more of the concentration of glucose, availability of oxygen, intensity of agitation, gas pressure, flow rate of supplied air or other gas mixture, viscosity of the culture, culture density, concentration of oxygen in the supplied air or other gas mixture, and temperature.

40. The method of any one of the **foregoing claims**, wherein the time between step (a) and step (b) is less than about 72 hours, less than about 48 hours, less than about 24 hours, less than about 12 hours, less than about 9 hours, less than about 6 hours, less than about 5 hours, less than

about 4 hours, less than about 3 hours, less than about 90 minutes, less than about 30 minutes, less than about 5 minutes, or less than about 1 minute.

41. The method of any one of the **foregoing claims**, wherein the time between step (b) and step (c) is less than about 10 hours, less than about 9 hours, less than about 8 hours, less than about 7 hours, less than about 6 hours, less than about 5 hours, less than about 4 hours, less than about 3 hours, less than about 2 hours, less than about 90 minutes, less than about 80 minutes, less than about 70 minutes, less than about 60 minutes, less than about 50 minutes, less than about 40 minutes, less than about 30 minutes, less than about 20 minutes, less than about 10 minutes, less than about 5 minutes, or less than about 1 minute.

42. The method of any one of the **foregoing claims**, wherein the culture of step (a) is produced by adding a carbon source to said culture, and culturing said culture until the carbon source is depleted.

43. The method of **claim 42**, wherein said carbon source comprises one or more of: glycerol, glucose, ethanol, citrate, sorbitol, xylose, trehalose, arabinose, galactose, fructose, melibiose, lactose, maltose, rhamnose, ribose, mannose, mannitol, and raffinose.

44. The method of **claim 42 or 43**, wherein the depletion of the carbon source is determined by detecting a decrease in the metabolic activity of said eukaryotic cells.

45. The method of **claim 44**, wherein said decrease in the metabolic activity of said eukaryotic cells is identified by detecting a decrease in the consumption of oxygen by said eukaryotic cells, by detecting an increase in pH in the culture, by detecting stabilization of the wet cell mass, or by detecting an increase in the concentration of ammonia in the culture.

46. The method of **claim 45**, wherein said decrease in the consumption of oxygen by said eukaryotic cells is identified by detecting an increase in the concentration of dissolved oxygen in said culture.

47. The method of any one of the **foregoing claims**, wherein said eukaryotic cells comprise yeast cells.

48. The method of **claim 47**, wherein said yeast cells comprise methylotrophic yeast.

49. The method of **claim 48**, wherein said methylotrophic yeast is of the genus *Pichia*.

50. The method of **claim 49**, wherein said methylotrophic yeast of the genus *Pichia* is *Pichia pastoris*.

51. The method of **claim 49**, wherein said methylotrophic yeast of the genus *Pichia* is selected from the group consisting of: *Pichia angusta*, *Pichia guillermordii*, *Pichia methanolica*, and *Pichia inositovera*.

52. The method of **claim 49**, wherein the genes that provide for expression of said multi-subunit complex are integrated into one or more genomic loci.

53. The method of claim 49, wherein at least one of said genomic loci are selected from the group consisting of the pGAP locus, 3' AOX TT locus; PpURA5; OCH1; AOX1; HIS4; GAP; pGAP; 3' AOX TT; ARG; and the HIS4 TT locus.

54. The method of any one of the **foregoing claims**, wherein at least one of the genes encoding said subunits of the multi-subunit complex are expressed under control of an inducible or constitutive promoter.

55. The method of **claim 54**, wherein said inducible promoter is selected from the group consisting of the AOX1, CUP1, tetracycline inducible, thiamine inducible, and FLD1 promoters.

56. The method of any one of the **foregoing claims**, wherein at least one of the genes encoding said subunits of the multi-subunit complex are expressed under control of a promoter selected from the group consisting of: the CUP1, AOX1, ICL1, glyceraldehyde-3-phosphate dehydrogenase (GAP), FLD1, ADH1, alcohol dehydrogenase II, GAL4, PHØ3, PHO5, and Pyk promoters, tetracycline inducible promoters, thiamine inducible promoters, chimeric promoters derived therefrom, yeast promoters, mammalian promoters, insect promoters, plant promoters, reptile promoters, amphibian promoters, viral promoters, and avian promoters.

57. The method of any one of the **foregoing claims**, wherein said eukaryotic cell is a diploid, tetraploid cell, or polyploid.

58. The method of any one of the **foregoing claims**, further comprising purifying said multi-subunit complex from said eukaryotic cells or from the culture medium.

59. The method of **claim 58**, wherein said multi-subunit complex is purified from an intracellular component, cytoplasm, nucleoplasm, or a membrane of said eukaryotic cells.

60. The method of **claim 58**, wherein said eukaryotic cells secrete said multi-subunit complex into the culture medium.

61. The method of **claim 60**, wherein said multi-subunit complex is purified from said culture medium.

62. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises a monospecific or bispecific antibody.

63. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises a human antibody or a humanized antibody or fragment thereof.

64. The method of **claim 63**, wherein said humanized antibody is of mouse, rat, rabbit, goat, sheep, or cow origin.

65. The method of **claim 62,** wherein said humanized antibody is of rabbit origin.

66. The method of any one of the **foregoing claims,** wherein said multi-subunit complex comprises a monovalent, bivalent, or multivalent antibody.

67. The method of **claim 4 or any one of claims 61 to 66,** wherein said antibody is purified from said culture by protein A and/or protein G affinity.

68. The method of any one of the **foregoing claims,** wherein at least one of the genes that provide for expression of a subunit of said multi-subunit complex in at least one of said eukaryotic cells in said panel is optimized for expression in said eukaryotic cell.

69. The method of any one of the **foregoing claims,** wherein said multi-subunit complex comprises an antibody and the purity of said antibody is assessed by measuring the fraction of the antibody produced by said eukaryotic cell that is contained in antibody complexes having the expected apparent hydrodynamic radius, is contained in antibody complexes having the expected molecular weight, and / or specifically binds a target of said antibody.

70. The method of any one of the **foregoing claims,** wherein said multi-subunit complex comprises an antibody and the yield of said antibody is assessed by determining the amount of antibody produced by said eukaryotic cell discounting any product-associated variants that are abnormally glycosylated, contained in antibody complexes other than complexes having the expected apparent hydrodynamic radius, contained in antibody complexes having the expected molecular weight, and / or that fail to specifically bind to the target of said antibody.

71. The method of claim 58 or 59, wherein the molecular weight of said antibody complexes is determined by non-reducing SDS-PAGE.

72. The method of any one of the **foregoing claims,** wherein said multi-subunit complex comprises an antibody, said method further comprising purifying said antibody.

73. The method of any one of the **foregoing claims**, wherein said culture cell produces a supernatant antibody titer of at least 100 mg / L, at least 150 mg / L, at least 200 mg / L, at least 250 mg / L, at least 300 mg / L, between 100 and 300 mg / L, between 100 and 500 mg / L, between 100 and 1000 mg / L, at least 1000 mg / L, at least 1250 mg/liter, at least 1500 mg/liter, at least about 1750 mg/liter, at least about 2000 mg/liter, at least about 10000 mg/liter, or more.

74. The method of any one of the **foregoing claims**, wherein one or more subunits of said multi-subunit complex are expressed from more than one gene copy.

75. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises an antibody which is expressed from between 1-10 copies of a gene encoding the light chain of said antibody and from 1-10 copies of a gene encoding the heavy chain of said antibody.

76. The method of any one of the **foregoing claims**, wherein genes that provide for expression of said multi-subunit complex are integrated into genome of said cells.

77. The method of any one of claims **1-75**, wherein genes that provide for expression of said multi-subunit complex are contained on an extrachromosomal element, plasmid, or artificial chromosome.

78. The method of **claim 76 or 77**, wherein said cells comprise more copies of the gene that provide for the expression of the light chain of said antibody than copies of the gene that provide for expression of the heavy chain of said antibody.

79. The method of **claim 76 or 76**, wherein the respective number of copies of the gene encoding the heavy chain of said antibody and the number of copies of the gene encoding the light chain of said antibody in said cells are: 2 and 2, 2 and 3, 3 and 3, 3 and 4, 3 and 5, 4 and 3, 4 and 4, 4 and 5, 4 and 6, 5 and 4, 5 and 5, 5 and 6, or 5 and 7.

80. The method of **claim 76 or 77**, wherein the respective number of copies of the gene encoding the heavy chain of said antibody and the number of copies of the gene encoding the light chain of said antibody in said cells are: 2 and 1, 3 and 1, 4 and 1, 5 and 1, 6 and 1, 7 and 1, 8 and 1, 9 and 1, 10 and 1, 1 and 2, 2 and 2, 3 and 2, 4 and 2, 5 and 2, 6 and 2, 7 and 2, 8 and 2, 9 and 2, 10 and 2, 1 and 3, 2 and 3, 3 and 3, 4 and 3, 5 and 3, 6 and 3, 7 and 3, 8 and 3, 9 and 3, 10 and 3, 1 and 4, 2 and 4, 3 and 4, 4 and 4, 5 and 4, 6 and 4, 7 and 4, 8 and 4, 9 and 4, 10 and 4, 1 and 5, 2 and 5, 3 and 5, 4 and 5, 5 and 5, 6 and 5, 7 and 5, 8 and 5, 9 and 5, 10 and 5, 1 and 6, 2 and 6, 3 and 6, 4 and 6, 5 and 6, 6 and 6, 7 and 6, 8 and 6, 9 and 6, 10 and 6, 1 and 7, 2 and 7, 3 and 7, 4 and 7, 5 and 7, 6 and 7, 7 and 7, 8 and 7, 9 and 7, 10 and 7, 1 and 8, 2 and 8, 3 and 8, 4 and 8, 5 and 8, 6 and 8, 7 and 8, 8 and 8, 9 and 8, 10 and 8, 1 and 9, 2 and 9, 3 and 9, 4 and 9, 5 and 9, 6 and 9, 7 and 9, 8 and 9, 9 and 9, 10 and 9, 1 and 10, 2 and 10, 3 and 10, 4 and 10, 5 and 10, 6 and 10, 7 and 10, 8 and 10, 9 and 10, 10 and 10.

81. The method of any one of the **foregoing claims**, wherein the culture of step (c) is grown in a production medium.

82. The method of **claim 81**, wherein said production medium is a minimal medium.

83. The method of **claim 81**, wherein said minimal medium lacks selective agents.

84. The method of **claim 81**, wherein said minimal medium lacks pre-formed amino acids or other complex biomolecules.

85. The method of **claim 81**, wherein the production medium is a complex medium.

86. The method of **claim 85**, wherein the complex medium comprises one or more of yeast extract, soy peptones, and other plant peptones.

87. The method of any one of the **foregoing claims**, wherein the culture of step (c) is grown to a high cell density.

88. The method of **claim 87**, wherein said high cell density is at least 50 g/L.

89. The method of **claim 87**, wherein said high cell density is at least 100 g/L.

90. The method of **claim 87**, wherein said high cell density is at least 300 g/L.

91. The method of **claim 87**, wherein said high cell density is at least 400 g/L.

92. The method of **claim 87**, wherein said high cell density is at least 500 g/L.

93. The method of **claim 87**, wherein said high cell density is at least 750 g/L.

94. The method of any one of the **foregoing claims**, wherein the yeast cells are cultured for at least 20 doublings and maintain high levels of expression of said multi-subunit complex after said at least 20 doublings.

95. The method of any one of the **foregoing claims**, wherein the cells of step (c) are cultured for at least 50 doublings and maintain high levels of expression of said multi-subunit complex after said at least 50 doublings.

96. The method of any one of the **foregoing claims**, wherein the cells of step (c) are cultured for at least 100 doublings and maintain high levels of expression of said multi-subunit complex after said at least 100 doublings.

97. The method of any one of the **foregoing claims**, wherein at least one subunit of said multi-subunit complex comprises a secretion signal.

98. The method of **claim 97**, wherein said multi-subunit complex comprises an antibody.

99. The method or composition of **claim 97 or 98**, wherein the secretion signal comprises one or more polypeptides selected from the group consisting of: SEQ ID NOS: 414 to 437 and any combination thereof.

100. The method of any one of the **foregoing claims**, wherein said multi-subunit complex is not any of the antibodies disclosed in U.S. Provisional Application No. 61/418,832, filed

December 1, 2010, PCT/US11/62963, filed December 1, 2011, U.S. Ser. No. 13/309,295, filed December 1, 2011, U.S. Ser. No. 13/309,153, filed December 1, 2011, U.S. Ser. No. 13/308,665 filed on December 1, 2011, and U.S. Ser. No. 13/308,831, filed December 1, 2011.

101. The method of any one of the foregoing claims, wherein said multi-subunit complex is not Ab1-NGF, Ab2-NGF, Ab3-NGF, Ab4-NGF, Ab5-NGF, Ab6-NGF, Ab7-NGF, Ab8-NGF, Ab9-NGF, Ab10-NGF, Ab11-NGF, Ab12-NGF, Ab13-NGF, Ab14-NGF, Ab15-NGF, Ab16-NGF, Ab17-NGF, Ab18-NGF, Ab19-NGF, Ab20-NGF, and Ab21-NGF, or an Fab2 or Fab1 fragment thereof.

102. The method of any one of the **foregoing claims**, wherein said multi-subunit complex does not contain at least one, at least two, at least three, at least four, at least five, or at least all six of the complementarity determining regions (CDRs) contained in any of the following antibodies: Ab1-NGF, Ab2-NGF, Ab3-NGF, Ab4-NGF, Ab5-NGF, Ab6-NGF, Ab7-NGF, Ab8-NGF, Ab9-NGF, Ab10-NGF, Ab11-NGF, Ab12-NGF, Ab13-NGF, Ab14-NGF, Ab15-NGF, Ab16-NGF, Ab17-NGF, Ab18-NGF, Ab19-NGF, Ab20-NGF, or Ab21-NGF and optionally having binding specificity for NGF.

103. The method of any one of the **foregoing claims**, wherein said multi-subunit complex does not comprise or consist of the light and heavy chain polypeptide sequences of SEQ ID NOs: 51 and 401, respectively, SEQ ID NOs: 53 and 402, respectively, SEQ ID NOs: 405 and 406, respectively, and SEQ ID NOs: 407 and 408, respectively.

104. The method of any one of the **foregoing claims**, wherein said multi-subunit complex does not comprise an antibody containing at least one, at least two, at least three, at least four, at least five, or at least all six of the CDRs of SEQ ID NOs: 55, 56, 57, 58, 59, and 60, and optionally having binding specificity for NGF.

105. The method of any one of the **foregoing claims**, wherein said multi-subunit complex does not comprise any of the antibodies or antibody coding sequences disclosed herein in the sections entitled "Anti-NGF Antibodies and Binding Fragments Thereof Having Binding Activity for NGF" and "Polynucleotides Encoding Anti-NGF Antibody Polypeptides."

[00181] Additionally, the present methods are not limited to production of multi-protein complexes but may also be readily adapted for use with ribonucleoprotein (RNP) complexes including telomerase, hnRNPs, Ribosomes, snRNPs, signal recognition particles, prokaryotic and eukaryotic RNase P complexes, and any other complexes that contain multiple distinct protein and / or RNA subunits. The host cell that expresses the multi-subunit complex may be produced by methods known in the art. For example, a panel of diploid or tetraploid yeast cells containing differing combinations of gene copy numbers may be generated by mating cells containing varying numbers of copies of the individual subunit genes (which numbers of copies preferably are known in advance of mating).

Definitions

[00182] As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the protein" includes reference to one or more proteins and equivalents thereof known to those skilled in the art, and so forth. All technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs unless clearly indicated otherwise.

[00183] *Bolus addition*: In the present disclosure, "bolus addition" generally refers to rapid change in concentration of a substance (such as ethanol) in contact with cultured cells (for example, in a culture medium). For example, the substance may be added to the cultured cells in a single addition, a succession of more than one addition, and/or infused over a period of time (e.g., over about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 90, or 120 minutes). The substance may also be added by replacing the culture medium in part or in full, for example by concentrating the cells (using centrifugation, filtration, settling, or other methods), removing part or all of the medium, and adding the substance, or by adding the cells to a medium containing the

[00943] Certain teachings related to methods for obtaining a clonal population of antigen-specific B cells were disclosed in U.S. Provisional patent application no. 60/801,412, filed May 19, 2006.

[00944] The disclosure of U.S. Provisional patent application no. 60/801,412 is herein incorporated by reference in its entirety.

[00945] Certain teachings related to humanization of rabbit-derived monoclonal antibodies and preferred sequence modifications to maintain antigen binding affinity were disclosed in International Application No. PCT/US2008/064421.

[00946] This corresponds to International Publication No. WO/2008/144757, entitled "Novel Rabbit Antibody Humanization Methods and Humanized Rabbit Antibodies".

[00947] This was filed May 21, 2008, the disclosure of which is herein incorporated by reference in its entirety.

[00948] Certain teachings related to producing antibodies or fragments thereof using mating competent yeast and corresponding methods were disclosed in U.S. Patent application no. 11/429,053, filed May 8, 2006, (U.S. Patent Application Publication No. US2006/0270045), the disclosure of which is herein incorporated by reference in its entirety.

[00949] The entire disclosure of each document cited herein (including patents, patent applications, journal articles, abstracts, manuals, books, or other disclosures), including each document cited in the Background, Summary, Detailed Description, and Examples, is hereby incorporated by reference herein in its entirety.

CLAIMS

1. A method of producing a multi-subunit complex, comprising:

   (a) providing a culture comprising yeast cells comprising genes that provide for the expression of the subunits of said multi-subunit complex;

   (b) adding a bolus of ethanol to said culture that results in a concentration of ethanol in said culture of between 0.2% and 2.75%; and

   (c) culturing said culture to produce said multi-subunit complex, wherein said culturing comprises adding a feed comprising a fermentable carbon source to said culture.

2. The method of **claim 1**, wherein the ethanol bolus enhances the formation of stable disulfide bonds relative to the same method effected in the absence of the bolus of ethanol.

3. The method of **claim 1**, wherein said multi-subunit complex contains one or more polypeptides comprising at least one disulfide bond.

4. The method of **claim 1**, wherein said multi-subunit complex comprises an antibody.

5. The method of any one of **claims 1 to 4**, which decreases the relative abundance of one or more product-associated variants relative to the same method effected in the absence of the bolus of ethanol.

6. The method of any one of **claims 1 to 4**, which decreases the relative abundance of product-associated variants having a higher or lower apparent molecular weight than said desired multi-subunit complex as detected by size exclusion chromatography or gel electrophoresis relative to the same method effected in the absence of the bolus of ethanol.

7. The method of any one of **claims 1 to 4**, which decreases the relative abundance of complexes having aberrant stoichiometry relative to the same method effected in the absence of the bolus of ethanol.

8. The method of any one of **claims 1 to 4**, which decreases the relative abundance of complexes having aberrant disulfide bonds relative to the same method effected in the absence of the bolus of ethanol.

9. The method of any one of **claims 1 to 4**, which decreases the relative abundance of complexes having reduced cysteines relative to the same method effected in the absence of the bolus of ethanol.

10. The method of any one of **claims 1 to 4**, which decreases the relative abundance of complexes having aberrant glycosylation relative to the same method effected in the absence of the bolus of ethanol.

11. The method of **claim 4**, which modulates the formation or stability of inter-heavy chain disulfide bonds.

12. The method of **claim 4 or 11**, which modulates the formation or stability of disulfide bonds linking the light and heavy chains.

13. The method of any one of **claims 4 or 11 to 12**, which decreases the relative abundance of one or more product-associated variants relative to the same method effected in the absence of the bolus of ethanol.

14. The method of **claim 13**, wherein said product-associated variants comprise one or more of the H1L1, H2L1, and H4L4 product-associate variants.

15. The method of any one of **claims 4 or 11 to 14**, which increases the purity of said antibody relative to said method effected in the absence of said bolus of ethanol.

16. The method of any one of the **foregoing claims**, wherein step (b) is effected prior to step (c).

17. The method of any one of **claims 1 to 15**, wherein step (b) is effected subsequent to step (c).

18. The method of any one of **claims 1 to 15**, wherein step (b) is effected concurrently with step (c).

19. The method of any one of the **foregoing claims**, wherein step (b) results in a concentration of ethanol in said culture of between 0.01% and 4% (w/v).

20. The method of any one of the **foregoing claims**, wherein step (b) results in a concentration of ethanol in said culture of between 0.4% and 2.25%, between 0.5% and 1.5%, between 0.5% and 2%, between 0.6% and 1.75%, between 0.7% and 1.5%, or between 0.8% and 1.25%.

21. The method of any one of the **foregoing claims**, wherein step (b) results in a concentration of ethanol in said culture that is at least 0.4%, 0.6%, 0.6%, 0.7%, 0.8% or 0.9% (w/v).

22. The method of any one of the **foregoing claims**, wherein step (b) results in a concentration of ethanol in said culture that is at most 2%, 1.8%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.35%, 0.3%, 0.25%, 0.2%, or 0.15% (w/v).

23. The method of any one of the **foregoing claims**, wherein step (b) comprises adding ethanol to said culture, adding a carrier comprising ethanol to said culture, adding said cells to a medium or carrier comprising ethanol, or replacing part of the culture medium.

24. The method of any one of the **foregoing claims**, wherein said bolus of ethanol is added to the culture medium over a period of time between 1 and 20 minutes.

25. The method of any one of the **foregoing claims**, wherein step (c) comprises providing oxygen to said cells.

26. The method of **claim 25**, wherein said providing oxygen comprises agitating said culture.

27. The method of **claim 25 or 26**, wherein said providing oxygen comprises contacting said culture with a gas mixture comprising oxygen.

28. The method of any one of the **foregoing claims**, wherein said fermentable carbon source comprises glucose or galactose.

29. The method of **claim 28**, wherein said feed comprises glucose.

30. The method of **claim 28**, wherein said feed comprises one or more of glucose, citrate, sorbitol, xylose, trehalose, arabinose, galactose, fructose, melibiose, lactose, maltose, rhamnose, ribose, mannose, mannitol, and raffinose.

31. The method of any one of the **foregoing claims**, further comprising maintaining the concentration of ethanol between an upper set point and a lower set point during step (c).

32. The method of **claim 31**, wherein said lower set point is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.2%, 0.3%, 0.4%, 0.6%, 0.6%, 0.7%, 0.8% or 0.9% (w/v).

33. The method of **claim 31 or 32**, wherein said upper set point is 4%, 3.5%, 3%, 2.5%, 2%, 1.8%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.35%, 0.3%, 0.25%, 0.2%, or 0.15% (w/v).

34. The method of **claim 31 or 32**, wherein said upper set point is at most 1.5%, 1.4%, 1.3, 1.2%, or 1.1% (w/v).

35. The method of any one of **claims 1 to 30**, further comprising maintaining the concentration of ethanol at a set point during step (c).

36. The method of **claim 35**, wherein said set point is 0.1%, 0.2%, 0. 3%, 0. 4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 01.%, 01.1%, 01.2%, 01.3%, 01.4%, or 01.5% (w/v).

37. The method of any one of **claims 1 to 30**, wherein step (c) comprises maintaining the concentration of ethanol in said culture between 0.01% and 4%, between 0.02% and 3.75%, between 0.04% and 3.5%, between 0.08% and 3.25%, between 0.1% and 3%, between 0.2% and 2.75%, between 0.3% and 2.5%, between 0.4% and 2.25%, between 0.5% and 2%, between 0.6% and 1.75%, between 0.7% and 1.5%, or between 0.8% and 1.25%.

38. The method of any one of claims 21 to 27, wherein the concentration of ethanol in said culture is maintained by controlling production of ethanol by said cells or by addition of ethanol to said culture.

39. The method of claim 38, wherein the step of controlling production of ethanol comprises controlling one or more of the concentration of glucose, availability of oxygen, intensity of agitation, gas pressure, flow rate of supplied air or other gas mixture, viscosity of the culture, culture density, concentration of oxygen in the supplied air or other gas mixture, and temperature.

40. The method of any one of the **foregoing claims**, wherein the time between step (a) and step (b) is less than 72 hours, less than 48 hours, less than 24 hours, less than 12 hours, less than 9 hours, less than 6 hours, less than 5 hours, less than 4 hours, less than 3 hours, less than 90 minutes, less than 30 minutes, less than 5 minutes, or less than 1 minute.

41. The method of any one of the **foregoing claims**, wherein the time between step (b) and step (c) is less than 10 hours, less than 9 hours, less than 8 hours, less than 7 hours, less than 6 hours, less than 5 hours, less than 4 hours, less than 3 hours, less than 2 hours, less than 90 minutes, less than 80 minutes, less than 70 minutes, less than 60 minutes, less than 50 minutes,

less than 40 minutes, less than 30 minutes, less than 20 minutes, less than 10 minutes, less than 5 minutes, or less than 1 minute.

42. The method of any one of the **foregoing claims**, wherein the culture of step (a) is produced by adding a carbon source to said culture, and culturing said culture until the carbon source is depleted.

43. The method of **claim 42**, wherein said carbon source comprises one or more of: glucose, citrate, sorbitol, xylose, trehalose, arabinose, galactose, fructose, melibiose, lactose, maltose, rhamnose, ribose, mannose, mannitol, and raffinose.

44. The method of **claim 42 or 43**, wherein the depletion of the carbon source is determined by detecting a decrease in the metabolic activity of said cells.

45. The method of **claim 44**, wherein said decrease in the metabolic activity of said cells is identified by detecting a decrease in the consumption of oxygen by said cells, by detecting an increase in pH in the culture, by detecting stabilization of the wet cell mass, or by detecting an increase in the concentration of ammonia in the culture.

46. The method of **claim 45**, wherein said decrease in the consumption of oxygen by said cells is identified by detecting an increase in the concentration of dissolved oxygen in said culture.

47. The method of any one of the **foregoing claims**, wherein said bolus of ethanol results in a concentration of ethanol in said culture of between between 1% and 1.5%.

48. The method of **claim 1**, wherein said yeast cells comprise methylotrophic yeast.

49. The method of **claim 48**, wherein said methylotrophic yeast is of the genus *Pichia*.

50. The method of **claim 49**, wherein said methylotrophic yeast of the genus *Pichia* is *Pichia pastoris*.

51. The method of **claim 49**, wherein said methylotrophic yeast of the genus *Pichia* is selected from the group consisting of: *Pichia angusta*, *Pichia guillermordii*, *Pichia methanolica*, and *Pichia inositovera*.

52. The method of **claim 49**, wherein the genes that provide for expression of said multi-subunit complex are integrated into one or more genomic loci.

53. The method of claim 49, wherein at least one of said genomic loci are selected from the group consisting of the pGAP locus, 3' AOX TT locus; PpURA5; OCH1; AOX1; HIS4; GAP; pGAP; 3' AOX TT; ARG; and the HIS4 TT locus.

54. The method of any one of the **foregoing claims**, wherein at least one of the genes encoding said subunits of the multi-subunit complex are expressed under control of an inducible or constitutive promoter.

55. The method of **claim 54**, wherein said inducible promoter is selected from the group consisting of the AOX1, CUP1, tetracycline inducible, thiamine inducible, and FLD1 promoters.

56. The method of any one of the **foregoing claims**, wherein at least one of the genes encoding said subunits of the multi-subunit complex are expressed under control of a promoter selected from the group consisting of: the CUP1, AOX1, ICL1, glyceraldehyde-3-phosphate dehydrogenase (GAP), FLD1, ADH1, alcohol dehydrogenase II, GAL4, PHO3, PHO5, and Pyk promoters, tetracycline inducible promoters, thiamine inducible promoters, chimeric promoters derived therefrom, yeast promoters, mammalian promoters, insect promoters, plant promoters, reptile promoters, amphibian promoters, viral promoters, and avian promoters.

57. The method of any one of the **foregoing claims**, wherein said cell is a diploid, tetraploid cell, or polyploid.

58. The method of any one of the **foregoing claims**, further comprising purifying said multi-subunit complex from said cells or from the culture medium.

59. The method of **claim 58**, wherein said multi-subunit complex is purified from an intracellular component, cytoplasm, nucleoplasm, or a membrane of said cells.

60. The method of **claim 58**, wherein said cells secrete said multi-subunit complex into the culture medium.

61. The method of **claim 60**, wherein said multi-subunit complex is purified from said culture medium.

62. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises a monospecific or bispecific antibody.

63. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises a human antibody or a humanized antibody or fragment thereof.

64. The method of **claim 63**, wherein said humanized antibody is of mouse, rat, rabbit, goat, sheep, or cow origin.

65. The method of **claim 63**, wherein said humanized antibody is of rabbit origin.

66. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises a monovalent, bivalent, or multivalent antibody.

67. The method of **claim 4 or any one of claims 61 to 66**, wherein said antibody is purified from said culture by protein A and/or protein G affinity.

68. The method of any one of the **foregoing claims**, wherein at least one of the genes that provide for expression of a subunit of said multi-subunit complex is optimized for expression in said cell.

69. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises an antibody and the purity of said antibody is assessed by measuring the fraction of the antibody produced by said cell that is contained in antibody complexes having the expected apparent hydrodynamic radius, is contained in antibody complexes having the expected molecular weight, and / or specifically binds a target of said antibody.

70. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises an antibody and the yield of said antibody is assessed by determining the amount of antibody produced by said cell discounting any product-associated variants that are abnormally glycosylated, contained in antibody complexes other than complexes having the expected apparent hydrodynamic radius, contained in antibody complexes having the expected molecular weight, and / or that fail to specifically bind to the target of said antibody.

71. The method of claim 69 or 70, wherein the molecular weight of said antibody complexes is determined by non-reducing SDS-PAGE.

72. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises an antibody, said method further comprising purifying said antibody.

73. The method of any one of the **foregoing claims**, wherein said culture cell produces a supernatant antibody titer of at least 100 mg / L, at least 150 mg / L, at least 200 mg / L, at least 250 mg / L, at least 300 mg / L, between 100 and 300 mg / L, between 100 and 500 mg / L, between 100 and 1000 mg / L, at least 1000 mg / L, at least 1250 mg/liter, at least 1500 mg/liter, at least 1750 mg/liter, at least 2000 mg/liter, at least 10000 mg/liter, or more.

74. The method of any one of the **foregoing claims**, wherein one or more subunits of said multi-subunit complex are expressed from more than one gene copy.

75. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises an antibody which is expressed from between 1-10 copies of a gene encoding the light chain of said antibody and from 1-10 copies of a gene encoding the heavy chain of said antibody.

76. The method of any one of the **foregoing claims**, wherein genes that provide for expression of said multi-subunit complex are integrated into genome of said cells.

77. The method of any one of claims **1-75**, wherein genes that provide for expression of said multi-subunit complex are contained on an extrachromosomal element, plasmid, or artificial chromosome.

78. The method of **claim 76 or 77**, wherein said cells comprise more copies of the gene that provide for the expression of the light chain of said antibody than copies of the gene that provide for expression of the heavy chain of said antibody.

79. The method of **claim 76 or 76**, wherein the respective number of copies of the gene encoding the heavy chain of said antibody and the number of copies of the gene encoding the light chain of said antibody in said cells are: 2 and 2, 2 and 3, 3 and 3, 3 and 4, 3 and 5, 4 and 3, 4 and 4, 4 and 5, 4 and 6, 5 and 4, 5 and 5, 5 and 6, or 5 and 7.

80. The method of **claim 76 or 77**, wherein the respective number of copies of the gene encoding the heavy chain of said antibody and the number of copies of the gene encoding the light chain of said antibody in said cells are: 2 and 1, 3 and 1, 4 and 1, 5 and 1, 6 and 1, 7 and 1, 8 and 1, 9 and 1, 10 and 1, 1 and 2, 2 and 2, 3 and 2, 4 and 2, 5 and 2, 6 and 2, 7 and 2, 8 and 2, 9 and 2, 10 and 2, 1 and 3, 2 and 3, 3 and 3, 4 and 3, 5 and 3, 6 and 3, 7 and 3, 8 and 3, 9 and 3, 10 and 3, 1 and 4, 2 and 4, 3 and 4, 4 and 4, 5 and 4, 6 and 4, 7 and 4, 8 and 4, 9 and 4, 10 and 4, 1 and 5, 2 and 5, 3 and 5, 4 and 5, 5 and 5, 6 and 5, 7 and 5, 8 and 5, 9 and 5, 10 and 5, 1 and 6, 2 and 6, 3 and 6, 4 and 6, 5 and 6, 6 and 6, 7 and 6, 8 and 6, 9 and 6, 10 and 6, 1 and 7, 2 and 7, 3

and 7, 4 and 7, 5 and 7, 6 and 7, 7 and 7, 8 and 7, 9 and 7, 10 and 7, 1 and 8, 2 and 8, 3 and 8, 4 and 8, 5 and 8, 6 and 8, 7 and 8, 8 and 8, 9 and 8, 10 and 8, 1 and 9, 2 and 9, 3 and 9, 4 and 9, 5 and 9, 6 and 9, 7 and 9, 8 and 9, 9 and 9, 10 and 9, 1 and 10, 2 and 10, 3 and 10, 4 and 10, 5 and 10, 6 and 10, 7 and 10, 8 and 10, 9 and 10, 10 and 10.

81. The method of any one of the **foregoing claims**, wherein the culture of step (c) is grown in a production medium.

82. The method of **claim 81**, wherein said production medium is a minimal medium.

83. The method of **claim 81**, wherein said minimal medium lacks selective agents.

84. The method of **claim 81**, wherein said minimal medium lacks pre-formed amino acids or other complex biomolecules.

85. The method of **claim 81**, wherein the production medium is a complex medium.

86. The method of **claim 85**, wherein the complex medium comprises one or more of yeast extract, soy peptones, and other plant peptones.

87. The method of any one of the **foregoing claims**, wherein the culture of step (c) is grown to a high cell density.

88. The method of **claim 87**, wherein said high cell density is at least 50 g/L.

89. The method of **claim 87**, wherein said high cell density is at least 100 g/L.

90. The method of **claim 87**, wherein said high cell density is at least 300 g/L.

91. The method of **claim 87**, wherein said high cell density is at least 400 g/L.

92. The method of **claim 87**, wherein said high cell density is at least 500 g/L.

93. The method of **claim 87**, wherein said high cell density is at least 750 g/L.

94. The method of any one of the **foregoing claims**, wherein the yeast cells are cultured for at least 20 doublings and maintain high levels of expression of said multi-subunit complex after said at least 20 doublings.

95. The method of any one of the **foregoing claims**, wherein the cells of step (c) are cultured for at least 50 doublings and maintain high levels of expression of said multi-subunit complex after said at least 50 doublings.

96. The method of any one of the **foregoing claims**, wherein the cells of step (c) are cultured for at least 100 doublings and maintain high levels of expression of said multi-subunit complex after said at least 100 doublings.

97. The method of any one of the **foregoing claims**, wherein at least one subunit of said multi-subunit complex comprises a secretion signal.

98. The method of **claim 97**, wherein said multi-subunit complex comprises an antibody.

99. The method or composition of **claim 97 or 98**, wherein the secretion signal comprises one or more polypeptides selected from the group consisting of: SEQ ID NOS: 414 to 437 and any combination thereof.

100. The method of any one of the foregoing claims, wherein said multi-subunit complex is not Ab1-NGF, Ab2-NGF, Ab3-NGF, Ab4-NGF, Ab5-NGF, Ab6-NGF, Ab7-NGF, Ab8-NGF, Ab9-NGF, Ab10-NGF, Ab11-NGF, Ab12-NGF, Ab13-NGF, Ab14-NGF, Ab15-NGF, Ab16-NGF, Ab17-NGF, Ab18-NGF, Ab19-NGF, Ab20-NGF, and Ab21-NGF, or an Fab2 or Fab1 fragment thereof.

101. The method of any one of the **foregoing claims**, wherein said multi-subunit complex does not contain at least one, at least two, at least three, at least four, at least five, or at least all six of the complementarity determining regions (CDRs) contained in any of the following

antibodies: Ab1-NGF, Ab2-NGF, Ab3-NGF, Ab4-NGF, Ab5-NGF, Ab6-NGF, Ab7-NGF, Ab8-NGF, Ab9-NGF, Ab10-NGF, Ab11-NGF, Ab12-NGF, Ab13-NGF, Ab14-NGF, Ab15-NGF, Ab16-NGF, Ab17-NGF, Ab18-NGF, Ab19-NGF, Ab20-NGF, or Ab21-NGF and optionally having binding specificity for NGF.

102. The method of any one of the **foregoing claims**, wherein said multi-subunit complex does not comprise or consist of the light and heavy chain polypeptide sequences of SEQ ID NOs: 51 and 401, respectively, SEQ ID NOs: 53 and 402, respectively, SEQ ID NOs: 405 and 406, respectively, and SEQ ID NOs: 407 and 408, respectively.

103. The method of any one of the **foregoing claims**, wherein said multi-subunit complex does not comprise an antibody containing at least one, at least two, at least three, at least four, at least five, or at least all six of the CDRs of SEQ ID NOs: 55, 56, 57, 58, 59, and 60, and optionally having binding specificity for NGF.