We claim:

1. A recombinant protein having N-acetylglucosaminyltransferase activity, wherein the recombinant protein catalyzes the transfer of N-acetylglucosamine to a terminal Manα3 residue and catalyzes the transfer of N-acetylglucosamine to a terminal Manα6 residue of an acceptor glycan, and ~~wherein the recombinant protein comprises a catalytic domain from at least two different enzymes~~

~~2.~~ ~~The recombinant protein of claim 1,~~ wherein the recombinant protein is a fusion protein comprising an N-acetylglucosaminyltransferase I catalytic domain and an N-acetylglucosaminyltransferase II catalytic domain.

~~3.~~2. The recombinant protein of claim ~~2~~1, wherein the N-acetylglucosaminyltransferase I catalytic domain and the N-acetylglucosaminyltransferase II catalytic domain are from human enzymes.

~~4.~~3. The recombinant protein of claim ~~3~~2, wherein the N-acetylglucosaminyltransferase I catalytic domain comprises a sequence that is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to amino acid residues 105-445 of SEQ ID NO: 1.

~~5.~~4. The recombinant protein of claim ~~3~~2 or claim 34, wherein the N-acetylglucosaminyltransferase II catalytic domain comprises a sequence that is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical amino acid residues 30-447 of SEQ ID NO: ~~21~~20.

~~6.~~5. The recombinant protein of any one of claims ~~2~~1-~~5~~4, wherein the N-acetylglucosaminyltransferase II catalytic domain is N-terminal to the N-acetylglucosaminyltransferase I catalytic domain.

~~7.~~6. The recombinant protein of any one of claims 12-~~6~~5, further comprising a spacer made of consecutive amino acids in between the N-acetylglucosaminyltransferase I catalytic domain and the N-acetylglucosaminyltransferase II catalytic domain.

~~8.~~7. The recombinant protein of claim ~~7~~6, wherein the spacer comprises sequence from a stem domain.

~~9.~~8. The recombinant protein of claim ~~7~~6 or claim ~~8~~7, wherein the spacer comprises a sequence selected from the group consisting of SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 122, and SEQ ID NO: 124.

~~10.~~9. The recombinant protein of claim ~~9~~8, wherein the spacer comprises a sequence selected from the group consisting of SEQ ID NO: 118, SEQ ID NO: 120, and SEQ ID NO: 124.

~~11.~~10. The recombinant protein of claim ~~9~~8, wherein the spacer comprises the sequence of SEQ ID NO: 124 or SEQ ID NO: 120.

~~12.~~11. The recombinant protein of claim ~~9~~8, wherein the spacer comprises the sequence of SEQ ID NO: 124.

~~13.~~12. The recombinant protein of any one of claims ~~2~~1-~~12~~11, further comprising a targeting peptide linked to the N-terminal end of the catalytic domains.

~~14.~~13. The recombinant protein of claim ~~13~~12, wherein the targeting peptide comprises a stem domain.

~~15.~~14. The recombinant protein of claim ~~8~~7 or claim ~~14~~13, wherein the stem domain is from a protein selected from the group consisting of a mannosidase, a mannosyltransferase, a glycosyltransferase, a Type 2 Golgi protein, MNN2, MNN4, MNN6, MNN9, MNN10, MNS1, KRE2, VAN1, and OCH1.

~~16.~~15. The recombinant protein of claim ~~15~~14, wherein the protein is from an organism selected from the group consisting of *Acremonium*, *Aspergillus*, *Aureobasidium*, *Cryptococcus*, *Chrysosporium*, *Chrysosporium lucknowense*, *Filibasidium*, *Fusarium*, *Gibberella*, *Humicola*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Myrothecium*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Piromyces*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, *Tolypocladium*, and *Trichoderma*.

~~17.~~16. The recombinant protein of claim ~~13~~12 or claim ~~14~~13, wherein the targeting peptide is a Kre2 targeting peptide.

~~18. The recombinant protein of any one of claims 14-17, wherein the targeting peptide further comprises a transmembrane domain linked to the N-terminal end of the stem domain.~~

~~19. The recombinant protein of any one of claims 14-17, wherein the targeting peptide further comprises a cytoplasmic domain linked to the N-terminal end of the stem domain.~~

~~20. The recombinant protein of claim 18 wherein the targeting peptide further comprises a cytoplasmic domain linked to the N-terminal end of the transmembrane domain.~~

~~21.~~17. A recombinant protein comprising a human N-acetylglucosaminyltransferase II catalytic domain and a human N-acetylglucosaminyltransferase I catalytic domain wherein the N-acetylglucosaminyltransferase II catalytic domain is located N-terminal to the N-acetylglucosaminyltransferase I catalytic domain, a spacer sequence comprising sequence from a human N-acetylglucosaminyltransferase I stem domain located in between the catalytic domains, and a targeting peptide located N-terminal to the N-acetylglucosaminyltransferase II catalytic domain wherein the targeting peptide comprises a cytoplasmic domain, a transmembrane domain, and a stem domain from human N-acetylglucosaminyltransferase II.

~~22.~~18. ~~A~~ The recombinant protein of Claim 1 comprising a sequence that is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 95.

~~23.~~19. A recombinant protein comprising a human N-acetylglucosaminyltransferase II catalytic domain and a N-acetylglucosaminyltransferase I catalytic domain, wherein the N-acetylglucosaminyltransferase II catalytic domain is located N-terminal to the N-acetylglucosaminyltransferase I catalytic domain; a spacer located in between the catalytic domains, wherein the spacer comprises sequence selected from the group consisting of SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 122, and SEQ ID NO: 124; and a targeting peptide located N-terminal to the N-acetylglucosaminyltransferase II catalytic domain wherein the targeting peptide comprises a cytoplasmic domain, a transmembrane domain, and a stem domain from human N-acetylglucosaminyltransferase II.

~~24.~~20. The recombinant protein of claim ~~23~~19, wherein the spacer comprises a sequence selected from the group consisting of SEQ ID NO: 118, SEQ ID NO: 120, and SEQ ID NO: 124.

~~25.~~21. The recombinant protein of claim ~~23~~19, wherein the spacer comprises the sequence of SEQ ID NO: 124 or SEQ ID NO: 120.

~~26.~~22. The recombinant protein of claim ~~23~~19, wherein the spacer comprises the sequence of SEQ ID NO: 124.

~~27.~~23. An isolated polynucleotide encoding the recombinant protein of any of the above claims.

~~28.~~24. An expression vector comprising the isolated polynucleotide of claim ~~27~~23 operably linked to a promoter.

~~29.~~25. A host cell comprising the expression vector of claim ~~28~~24.

~~30.~~26. A method of producing a complex N-glycan, comprising :

(1) providing a host cell, wherein the host cell comprises a polynucleotide encoding a fusion protein ~~comprising an N-acetylglucosaminyltransferase I catalytic domain and an N-acetylglucosaminyltransferase II catalytic domain~~of Claim 1; and
(2) culturing the host cell such that the fusion protein is expressed, wherein the fusion protein catalyzes the transfer of N-acetylglucosamine to a terminal Manα3 residue and N-acetylglucosamine to a terminal Manα6 residue of an acceptor glycan to produce a complex N-glycan.

~~31.~~27. The method of claim ~~30~~26, wherein the acceptor glycan is attached to a heterologous polypeptide.

~~32.~~28. The method of claim ~~30~~26 or claim ~~31~~27, wherein the complex N-glycan is GlcNAcβ2Manβ3(GlcNAcβ2Manβ6)Manβ4GlcNAcβ4GlcNAc.

~~33.~~29. The method of any one of claims ~~30~~26-~~32~~28, wherein the host cell is a filamentous fungal cell selected from the group consisting of *Trichoderma* sp., *Acremonium*, *Aspergillus*, *Aureobasidium*, *Cryptococcus*, *Chrysosporium*, *Chrysosporium lucknowense*, *Filibasidium*, *Fusarium*, *Gibberella*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Myrothecium*, *Neocallimastix*,

Neurospora, *Paecilomyces*, *Penicillium*, *Piromyces*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, and *Tolypocladium*.

~~34.~~30. The method of any one of claims ~~30~~26-~~33~~29, wherein the host cell further comprises a polynucleotide encoding a UDP-GlcNAc transporter.

~~35.~~31. The method of any one of claims ~~30-34~~26-30, wherein the host cell has a reduced level of activity of a dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase compared to the level of activity in a wild-type host cell.

~~36.~~32. The method of claim ~~35~~31, wherein the host cell has a reduced level of expression of an *alg3* gene compared to the level of expression in a wild-type host cell.

~~37.~~33. The method of claim ~~36~~32, wherein the *alg3* gene is deleted from the host cell.

~~38.~~34. The method of any one of claims ~~30-37~~26-33, wherein the host cell has a reduced level of activity of an α-1,6-mannosyltransferase as encoded by an och1 gene, compared to the level of activity in a wild-type host cell.

~~39.~~35. The method of claim ~~38~~34, wherein the host cell has a reduced level of expression of an och1 gene compared to the level of expression in a wild-type host cell.

~~40.~~36. The method of any one of claims ~~30-39~~26-35, wherein the host cell further comprises a polynucleotide encoding an α-1,2-mannosidase.

~~41.~~37. The method of any one of claims ~~30-40~~26-36, wherein the host cell further comprises a polynucleotide encoding a β-1,4-galactosyltransferase.

~~42.~~38. The method of any one of claims ~~30-41~~26-37, wherein the host cell further comprises a polynucleotide encoding a sialyltransferase.

~~43.~~39. A method of producing a complex N-glycan, comprising :

(1) providing a *Trichoderma* host cell, wherein the host cell has a reduced level of expression of an *alg3* gene compared to the level of expression in a wild-type host cell and comprises ~~a first polynucleotide encoding an N-acetylglucosaminyltransferase I catalytic domain and a second polynucleotide encoding an N-acetylglucosaminyltransferase II catalytic domain~~a recombinant protein of Claim 1; and,

(2) culturing the host cell to produce a complex N-glycan.

~~44. A method of producing a complex N-glycan, comprising incubating a fusion protein comprising an N-acetylglucosaminyltransferase I catalytic domain and an N-acetylglucosaminyltransferase II catalytic domain, an acceptor glycan, and an N-acetylglucosamine donor together in a buffer, wherein the fusion protein catalyzes the transfer of N-acetylglucosamine to a terminal Manα3 residue and N-acetylglucosamine to a terminal Manα6 residue of an acceptor glycan to produce a complex N-glycan.~~

~~45. A filamentous fungal cell comprising a mutation of *alg3* and Man3GlcNAc2, wherein the Man3GlcNAc2 comprises at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% of neutral N-glycans in the cell.~~

~~46. The filamentous fungal cell of claim 45, wherein the mutation of *alg3* is a deletion of *alg3*.~~

~~47. The filamentous fungal cell of claim 45 or claim 46, wherein the cell is a Trichoderma reesei cell.~~

~~48. The filamentous fungal cell of any one of claims 45-47, further comprising a first polynucleotide encoding an N-acetylglucosaminyltransferase I catalytic domain and a second polynucleotide encoding an N-acetylglucosaminyltransferase II catalytic domain.~~

~~49. The filamentous fungal cell of any one of claims 45-47, further comprising a polynucleotide encoding a fusion protein comprising an N-acetylglucosaminyltransferase I catalytic domain and an N-acetylglucosaminyltransferase II catalytic domain.~~

~~50. A method of producing a Man3GlcNAc2 glycan in a host cell, comprising:~~

> ~~(1) providing a host cell with a reduced level of activity of a mannosyltransferase compared to the level of activity in a wild-type host cell; and~~
> ~~(2) culturing the host cell to produce a Man3GlcNAc2 glycan, wherein the Man3GlcNAc2 glycan comprises at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% of the neutral N-glycans in the host cell.~~

~~51. The method of claim 50, wherein the Man3GlcNAc2 glycan is attached to a heterologous polypeptide~~

~~52. The method of claim 50 or claim 51, wherein the mannosyltransferase is a dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase.~~

~~53. The method of any one of claims 50-52, wherein the host cell has a reduced level of expression of an *alg3* gene compared to the level of expression in a wild-type host cell.~~

~~54. The method of any one of claims 50-53, wherein the host cell is a *Trichoderma* cell.~~

~~55. The method of any one of claims 50-54, wherein the level of activity of alpha-1,6-mannosyltransferase in the host cell is not reduced compared to the level of activity in a wild-type host cell.~~

~~56. The method of any one of claims 50-55, wherein the host cell comprises an endogenous polynucleotide encoding an α-1,2-mannosidase.~~

~~57.~~40. A filamentous fungal cell having a reduced level of expression of an *alg3* gene compared to the level of expression in a wild-type filamentous fungal cell, wherein the filamentous fungal cell comprises a recombinant protein according to any one of claims 1-18~~22~~.

~~58.~~41. The filamentous fungal cell of claim ~~57~~40, wherein the *alg3* gene comprises a mutation.

59.42. The filamentous fungal cell of claim 5841, wherein the mutation of the *alg3* gene is a deletion of the *alg3* gene.

60.43. The filamentous fungal cell of any one of claims 57-5940-42, wherein the fusion protein is encoded by a polynucleotide operably linked to a promoter.

61.44. The filamentous fungal cell of claim 6043, wherein the promoter is an inducible promoter.

62.45. The filamentous fungal cell of claim 6144, wherein the inducible promoter is the *cbh1* promoter.

63.46. The filamentous fungal cell of any one of claims 57-6240-45, wherein the filamentous fungal cell further comprises a polynucleotide encoding a UDP-GlcNAc transporter.

64.47. The filamentous fungal cell of any one of claims 57-6340-46, wherein the filamentous fungal cell has a reduced level of activity of an α-1,6-mannosyltransferase as encoded by an *och1* gene, as compared to the level of activity in a wild-type filamentous fungal cell.

65.48. The filamentous fungal cell of claim 6447, wherein the filamentous fungal cell has a reduced level of expression of an och1 gene compared to the level of expression in a wild-type filamentous fungal cell.

66.49. The filamentous fungal cell of any one of claims 57-6540-48, wherein the filamentous fungal cell further comprises a polynucleotide encoding an α-1,2-mannosidase.

67.50. The filamentous fungal cell of any one of claims 57-6640-49, wherein the filamentous fungal cell further comprises a polynucleotide encoding a β-1,4-galactosyltransferase.

68.51. The filamentous fungal cell of any one of claims 57-6740-50, wherein the filamentous fungal cell further comprises a polynucleotide encoding a sialyltransferase.

69.52. The filamentous fungal cell of any one of claims 57-6840-51, wherein the filamentous fungal cell is selected from the group consisting of *Trichoderma* sp., *Acremonium*, *Aspergillus*, *Aureobasidium*, *Cryptococcus*, *Chrysosporium*, *Chrysosporium lucknowense*, *Filibasidium*, *Fusarium*, *Gibberella*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Myrothecium*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Piromyces*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, and *Tolypocladium*.