

WHAT IS CLAIMED IS:

1. A monoclonal population of multimeric binding molecules, each binding molecule comprising ten or twelve IgM-derived heavy chains, wherein the IgM-derived heavy chains comprise glycosylated IgM heavy chain constant regions each associated with a binding domain that specifically binds to a target, wherein each IgM heavy chain constant region comprises at least one, at least two, at least three, at least four, or at least five asparagine (N)-linked glycosylation motifs, wherein an N-linked glycosylation motif comprises the amino acid sequence N-X₁-S/T, wherein N is asparagine, X₁ is any amino acid except proline, and S/T is serine or threonine, wherein at least one, at least two, or at least three of the N-linked glycosylation motifs on each IgM heavy chain constant region are occupied by a complex glycan, and wherein the monoclonal population of binding molecules comprises at least thirty-five (35), at least 40, at least 45, at least 50, at least 55, at least 60, or at least 65 moles sialic acid per mole of the binding molecules.
2. The monoclonal population of binding molecules of claim 1, comprising about 40 to about 70, about 40 to about 60, about 40 to about 55, about 40 to about 50, about 50 to about 70, about 60 to about 70 moles sialic acid per mole of binding molecule.
3. The monoclonal population of binding molecules of claim 1, wherein the IgM heavy chain constant regions are human IgM heavy chain constant regions or variants thereof comprising five N-linked glycosylation motifs N-X₁-S/T starting at amino acid positions corresponding to amino acid 46 (motif N1), amino acid 209 (motif N2), amino acid 272 (motif N3), amino acid 279 (motif N4), and amino acid 440 (motif N5) of SEQ ID NO: 1 (alleleIGHM*03) or SEQ ID NO: 2 (alleleIGHM*04).
4. The monoclonal population of binding molecules of claim 1, produced by the method of cell line modification, *in vitro* glycoengineering, or any combination thereof.
5. The monoclonal population of binding molecules of claim 4, wherein the cell line modification comprises transfecting a cell line that produces the monoclonal population of binding molecules with a gene encoding a sialyltransferase, thereby producing a modified cell line that overexpresses the sialyltransferase.
6. The monoclonal population of binding molecules of claim 4, wherein *in vitro* glycoengineering comprises contacting the monoclonal population of binding molecules with a soluble sialyltransferase and a sialic acid substrate.

7. The monoclonal population of binding molecules of claim 6, wherein the sialyltransferase comprises a soluble variant of human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) (SEQ ID NO: 3) and/or the sialic acid substrate comprises cytidine monophosphate-N-acetyl-neuraminic acid (CMP-NANA).

8. The monoclonal population of binding molecules of claim 6, wherein the mass ratio of binding molecule: sialic acid substrate is about 1:4 to about 40:1 and/or the mass ratio of binding molecule: sialyltransferase is about 80:1 to about 5000:1.

9. The monoclonal population of binding molecules of claim 6, wherein the contacting of the monoclonal population of binding molecules with the soluble sialyltransferase and the sialic acid substrate comprises at least 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 24 hours, 36 hours, or 48 hours of contact at about 2° C to about 40° C.

10. The monoclonal population of binding molecules of claim 1, wherein each binding molecule is a pentameric or a hexameric IgM antibody comprising five or six bivalent IgM binding units, respectively, wherein each binding unit comprises two IgM heavy chains each comprising a VH situated amino terminal to the variant IgM constant region, and two immunoglobulin light chains each comprising a light chain variable domain (VL) situated amino terminal to an immunoglobulin light chain constant region, and wherein the VH and VL combine to form an antigen-binding domain that specifically binds to the target.

11. The monoclonal population of binding molecules of claim 10, wherein each binding molecule is pentameric and further comprises a J-chain, or functional fragment thereof, or a functional variant thereof.

12. The monoclonal population of binding molecules of claim 11, wherein the J-chain is a mature human J-chain comprising the amino acid sequence SEQ ID NO: 6 or a functional fragment thereof, or a functional variant thereof.

13. The monoclonal population of binding molecules of claim 11, wherein the variant J-chain or functional fragment thereof comprises an amino acid substitution at the amino acid position corresponding to amino acid Y102 of the wild-type mature human J-chain of SEQ ID NO: 6.

14. The monoclonal population of binding molecules of claim 13, wherein the amino acid corresponding to Y102 of SEQ ID NO: 6 is substituted with alanine (A).

15. The monoclonal population of binding molecules of claim 14, wherein the J-chain comprises the amino acid sequence SEQ ID NO: 7.

16. The monoclonal population of binding molecules of claim 11, wherein the J-chain or fragment or variant thereof is a modified J-chain further comprising a heterologous moiety, wherein the heterologous moiety is fused or conjugated to the J-chain or fragment or variant thereof.

17. The monoclonal population of binding molecules of claim 16, wherein the heterologous moiety is a polypeptide fused to the J-chain or fragment or variant thereof.

18. The monoclonal population of binding molecules of claim 17, wherein the heterologous polypeptide is fused to the J-chain or fragment or variant thereof via a peptide linker comprising at least 5 amino acids, but no more than 25 amino acids.

19. The monoclonal population of binding molecules of claim 17, wherein the heterologous polypeptide is fused to the N-terminus of the J-chain or fragment or variant thereof, to the C-terminus of the J-chain or fragment or variant thereof, or to both the N-terminus and C-terminus of the J-chain or fragment or variant thereof, wherein the heterologous polypeptides fused to both the N-terminus and C-terminus can be the same or different.

20. The monoclonal population of binding molecules of claim 17, wherein the heterologous polypeptide comprises a scFv fragment.

21. The monoclonal population of binding molecules of claim 20, wherein the heterologous scFv fragment binds to CD3ε.

22. A pharmaceutical composition comprising the monoclonal population of binding molecules of any one of claims 1 to 21 and a pharmaceutically acceptable excipient.

23. A recombinant host cell that produces the monoclonal population of binding molecules of any one of claims 1 to 21.

24. A method of producing the monoclonal population of binding molecules of any one of claims 1 to 21, comprising culturing the host cell of claim 22, and recovering the population of binding molecules.

25. A method for producing a monoclonal population of highly sialylated multimeric binding molecules, comprising providing a cell line that expresses the monoclonal population of binding molecules, culturing the cell line, and recovering the monoclonal population of binding molecules, wherein each binding molecule comprises ten or twelve IgM-derived heavy chains, wherein the IgM-derived heavy chains comprise glycosylated IgM heavy chain constant regions each associated with a binding domain that specifically binds to a target, wherein each IgM heavy chain constant region comprises at least three, at

least four, or at least five asparagine(N)-linked glycosylation motifs, wherein an N-linked glycosylation motif comprises the amino acid sequence N-X₁-S/T, wherein N is asparagine, X₁ is any amino acid except proline, and S/T is serine or threonine, wherein on average at least one, at least two, or at least three of the N-linked glycosylation motifs on each IgM heavy chain constant region in the population are occupied by complex glycans, and wherein the cell line, recovery process, or a combination thereof is optimized to enrich for complex glycans comprising at least one, two, three, or four sialic acid terminal monosaccharides per glycan.

26. The method of claim 25, wherein the cell line, recovery process, or a combination thereof is optimized to result in a monoclonal population of binding molecules comprising at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, or at least 65 moles sialic acid per mole of binding molecule; or comprising about 40 to about 70, about 40 to about 60, about 40 to about 55, about 40 to about 50, about 50 to about 70, about 60 to about 70 moles sialic acid per mole of binding molecule.

27. The method of claim 25, wherein the IgM heavy chain constant regions are derived from human IgM heavy chain constant regions comprising five N-linked glycosylation motifs N-X₁-S/T starting at amino acid positions corresponding to amino acid 46 (motif N1), amino acid 209 (motif N2), amino acid 272 (motif N3), amino acid 279 (motif N4), and amino acid 440 (motif N5) of SEQ ID NO: 1 (allele IGHM*03) or SEQ ID NO: 2 (allele IGHM*04).

28. The method of claim 25, wherein the provided cell line is modified to overexpress a sialyltransferase.

29. The method of claim 25, wherein the recovery process comprises subjecting the monoclonal population of binding molecules to *in vitro* glycoengineering, wherein the *in vitro* glycoengineering comprises contacting the monoclonal population of binding molecules with a soluble sialyltransferase and a sialic acid substrate.

30. The method of claim 29, wherein the sialyltransferase comprises a soluble variant of human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) (SEQ ID NO: 3) and/or the sialic acid substrate comprises cytidine monophosphate (CMP)-N-acetyl-neuraminic acid (CMP-NANA).

31. The method of claim 29, wherein the mass ratio of binding molecule: sialic acid substrate is about 1:4 to about 40:1 and/or the mass ratio of binding molecule: sialyltransferase is about 80:1 to about 5000:1.

32. The method of any one of claims 29 to 31, wherein the contacting of the monoclonal population of binding molecules with the soluble sialyltransferase and the sialic acid substrate comprises at least 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 24 hours, 36 hours, or 48 hours of contact at about 2° C to about 40° C.