

Sugar moieties and their derivatives covalently linked to specific residues on an antibody can determine how they are recognized by receptors such as asialo-glycoprotein (ASGP) receptor, which in turn determines how quickly they are cleared from systemic circulation. Each IgM heavy chain constant region has five sites of asparagine- (N-)linked glycosylation, and the J-chain has one N-linked glycosylation site. Thus, a pentameric, J-chain containing IgM contains up to 51 glycan moieties, which results in a complex glycosylation profile (Hennicke, J., *et al.*, *Anal. Biochem.* 539:162-166 (2017)). The complexity of glycans can make manufacture of homogenously glycosylated material difficult.

[0006] Despite the advances made in the design of multimeric antibodies, there remains a need to be able to manipulate the physical, pharmacokinetic and pharmacodynamic properties of these molecules.

SUMMARY

[0007] Provided herein is a monoclonal population of multimeric binding molecules, each binding molecule including ten or twelve IgM-derived heavy chains, where the IgM-derived heavy chains include glycosylated IgM heavy chain constant regions each associated with a binding domain that specifically binds to a target, where each IgM heavy chain constant region includes at least one, at least two, at least three, at least four, or at least five asparagine (N)-linked glycosylation motifs, where an N-linked glycosylation motif includes the amino acid sequence N-X₁-S/T, where N is asparagine, X₁ is any amino acid except proline, and S/T is serine or threonine, where 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 where the monoclonal population of binding molecules includes at least thirty-five (35) moles sialic acid per mole of the binding molecules. In one aspect, the present disclosure provides 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.

[0008] In some embodimentexamples, the monoclonal population of binding molecules includes at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 124, at least 130, at least 140, or at least 146 moles sialic acid per mole of binding molecule. In some embodimentexamples, the monoclonal population of binding molecules includes 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 binding molecule. In some embodimentexamples, the monoclonal population of binding molecules includes 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.

[0009] In some [embodimentexamples](#), the IgM heavy chain constant regions are human IgM heavy chain constant regions or variants thereof including 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). In some [embodimentexamples](#), motifs N1, N2, and N3 are occupied by complex glycans.

[0010] In some [embodimentexamples](#), the monoclonal population of binding molecules is produced by the method of cell line modification, *in vitro* glycoengineering, or any combination thereof.

[0011] In some [embodimentexamples](#), the cell line modification includes 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. In some [embodimentexamples](#), the sialyltransferase includes human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) (SEQ ID NO: 3). In some [embodimentexamples](#), the cell line modification further includes transfecting a cell line that produces the monoclonal population of binding molecules with a gene encoding a galactosyltransferase, thereby producing a modified cell line that overexpresses the galactosyltransferase. In some [embodimentexamples](#), the galactosyltransferase includes human beta-1,4-galactosyltransferase 4 (B4GALT4) (SEQ ID NO: 4).

[0012] In some [embodimentexample](#)s, the *in vitro* glycoengineering includes contacting the monoclonal population of binding molecules with a soluble sialyltransferase and a sialic acid substrate. In some [embodimentexample](#)s, the sialyltransferase includes a soluble variant of human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) (SEQ ID NO: 3). In some [embodimentexample](#)s, the soluble variant of ST6GAL1 includes amino acids x to 406 of SEQ ID NO: 3, where x is an integer from 27 to 120. In some [embodimentexamples](#), the soluble variant of ST6GAL1 includes amino acids 120 to 406, 115 to 406, 110 to 406, 109 to 406, 105 to 406, 100 to 406, 95 to 406, 90 to 406, 89 to 406, 88 to 406, 87 to 406, 86 to 406, 85 to 406, 84 to 406, 83 to 406, 82 to 406, 81 to 406, 80 to 406, 75 to 406, 70 to 406, 65 to 406, 60 to 406, 55 to 406, 50 to 406, 45 to 406, 40 to 406, 35 to 406, 30 to 406, or 27 to

406 of SEQ ID NO: 3. In some [embodimentexamples](#), the sialic acid substrate includes cytidine monophosphate-N-acetyl-neuraminic acid (CMP-NANA).

[0013] In some [embodimentexamples](#), the mass ratio of binding molecule: sialic acid substrate is about 1:4 to about 40:1. In some [embodimentexamples](#), the mass ratio of binding molecule: sialyltransferase is about 80:1 to about 5000:1. In some [embodimentexamples](#), the mass ratio of binding molecule: sialyltransferase is about 500:1. In some [embodimentexamples](#), the mass ratio of binding molecule: sialic acid substrate: sialyltransferase is about 500:62.5:1. In some [embodimentexamples](#), the mass ratio of binding molecule: sialyltransferase is about 2000:1. In some [embodimentexamples](#), the mass ratio of binding molecule: sialic acid substrate: sialyltransferase is about 2000:500:1. In some [embodimentexamples](#), the molar ratio of binding molecule: sialyltransferase is about 80:1. In some [embodimentexamples](#), the molar ratio of binding molecule: sialic acid substrate: sialyltransferase is about 80:500:1.

[0014] In some [embodimentexamples](#), the contacting of the monoclonal population of binding molecules with the soluble sialyltransferase and the sialic acid substrate comprises at least 30 minutes of contact. In some [embodimentexamples](#), the contacting comprises at least 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 24 hours, 36 hours, or 48 hours of contact. In some [embodimentexamples](#), the contacting of the monoclonal population of binding molecules with the soluble sialyltransferase and the sialic acid substrate occurs at about 2° C to about 40° C. In some [embodimentexamples](#), the contacting occurs at 15° C to about 37° C, 15° C to about 30° C, or 15° C to about 25° C.

[0015] In some [embodimentexamples](#), the *in vitro* glycoengineering further includes contacting the monoclonal population of binding molecules with a galactosyltransferase and a galactose substrate. In some [embodimentexamples](#), the galactosyltransferase includes a soluble variant of human beta-1,4-galactosyltransferase 4 (B4GALT4) (SEQ ID NO: 4). In some [embodimentexamples](#), the soluble variant of B4GALT4 includes amino acids x to 344 of SEQ ID NO: 4, where x is an integer from 39 to 120. In some [embodimentexamples](#), the soluble variant of B4GALT4 includes amino acids 120 to 344, 115 to 344, 110 to 344, 105 to 344, 100 to 344, 95 to 344, 90 to 344, 85 to 344, 80 to 344, 75 to 344, 70 to 344, 65 to 344, 60 to 344, 55 to 344, 50 to 344, 45 to 344, 40 to 344, or 39 to 344 of SEQ ID NO: 4. In some [embodimentexamples](#), the galactose substrate includes uridine-diphosphate- α -D-galactose (UDP-Gal). In some [embodimentexamples](#), the contacting with the galactosyltransferase and the galactose substrate occurs prior to or simultaneously with the contacting with the soluble sialyltransferase and sialic acid substrate.

- [0016] In some embodimentexamples, each binding molecule is multispecific, and two or more binding domains associated with the IgM heavy chain constant regions of each binding molecule specifically bind to different targets. In some embodimentexamples, the binding domains associated with the IgM heavy chain constant regions of each binding molecule specifically bind to the same target. In some embodimentexamples, the binding domains associated with the IgM heavy chain constant regions of each binding molecule are identical.
- [0017] In some embodimentexamples, the binding domains are antibody-derived antigen-binding domains. In some embodimentexamples, each binding molecule is a pentameric or a hexameric IgM antibody including five or six bivalent IgM binding units, respectively, where each binding unit includes two IgM heavy chains each including a VH situated amino terminal to the variant IgM constant region, and two immunoglobulin light chains each including a light chain variable domain (VL) situated amino terminal to an immunoglobulin light chain constant region, and where the VH and VL combine to form an antigen-binding domain that specifically binds to the target. In some embodimentexamples, each antigen-binding domain of each binding molecule binds to the same target. In some embodimentexamples, each antigen-binding domain of each binding molecule is identical.
- [0018] In some embodimentexamples, the target is a target epitope, a target antigen, a target cell, a target organ, or a target virus.
- [0019] In some embodimentexamples, each binding molecule is pentameric and further includes a J-chain, or functional fragment thereof, or a functional variant thereof. In some embodimentexamples, the J-chain is a mature human J-chain including the amino acid sequence SEQ ID NO: 6 or a functional fragment thereof, or a functional variant thereof. In some embodimentexamples, the J-chain includes an N-linked glycosylation motif N-X₁-S/T starting at amino acid positions corresponding to amino acid 49 of SEQ ID NO: 6 (motif N6).
- [0020] In some embodimentexamples, the J-chain is a functional variant J-chain including one or more single amino acid substitutions, deletions, or insertions relative to a reference J-chain identical to the variant J-chain except for the one or more single amino acid substitutions, deletions, or insertions, and where the monoclonal population of binding molecules exhibits an increased serum half-life upon administration to a subject animal relative to a reference IgM-derived binding molecule that is identical except for the one or more single amino acid substitutions, deletions, or insertions in the variant J-chain, and is administered using the same method to the same animal species. In some embodimentexamples, the variant J-chain or functional fragment thereof includes one, two, three, or four single amino acid

substitutions, deletions, or insertions relative to the reference J-chain. In some [embodimentexamples](#), the variant J-chain or functional fragment thereof includes 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.

[0021] In some [embodimentexamples](#), the amino acid corresponding to Y102 of SEQ ID NO: 6 is substituted with alanine (A). In some [embodimentexamples](#), the J-chain includes the amino acid sequence SEQ ID NO: 7.

[0022] In some [embodimentexamples](#), the J-chain or fragment or variant thereof is a modified J-chain further including a heterologous moiety, where the heterologous moiety is fused or conjugated to the J-chain or fragment or variant thereof. In some [embodimentexamples](#), the heterologous moiety is a polypeptide fused to the J-chain or fragment or variant thereof. In some [embodimentexamples](#), the heterologous polypeptide is fused to the J-chain or fragment or variant thereof via a peptide linker. In some [embodimentexamples](#), the peptide linker includes at least 5 amino acids, but no more than 25 amino acids. In some [embodimentexamples](#), the peptide linker consists of GGGGSGGGGSGGGGS (SEQ ID NO: 43).

[0023] In some [embodimentexamples](#), the heterologous polypeptide is fused to the N-terminus of the J-chain or fragment or variant thereof or to the C-terminus of the J-chain or fragment or variant thereof. In some [embodimentexamples](#), heterologous moieties that can be the same or different are fused to the N-terminus and C-terminus of the J-chain or fragment or variant thereof.

[0024] In some [embodimentexamples](#), the heterologous polypeptide includes a binding domain. In some [embodimentexamples](#), the binding domain of the heterologous polypeptide is an antibody or antigen-binding fragment thereof. In some [embodimentexamples](#), the antigen-binding fragment is a scFv fragment. In some [embodimentexamples](#), the heterologous scFv fragment binds to CD3 ϵ . In some [embodimentexamples](#), the modified J-chain includes the amino acid sequence SEQ ID NO: 36 (V15J), SEQ ID NO: 37 (V15J*), SEQ ID NO: 38 (SJ*), SEQ ID NO: 31 (A-55-J*), SEQ ID NO: 32 (A-56-J*), SEQ ID NO: 33 (A-57-J*), amino acids 20-420 of SEQ ID NO: 34 (VJH), amino acids 20-420 of SEQ ID NO: 35 (VJ*H), or SEQ ID NOs: 6 or 7 fused via a peptide linker to an anti-CD3 ϵ scFv including HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 amino acid sequences including SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 20, and SEQ ID NO: 21, respectively.

[0025] ~~Also provided herein~~In another aspect, the present disclosure provides is a pharmaceutical composition comprising the monoclonal population of binding molecules disclosed herein and a pharmaceutically acceptable excipient.

[0026] ~~In yet another aspect, the present disclosure provides~~ ~~Also provided herein is~~ a recombinant host cell that produces the monoclonal population of binding molecules disclosed herein.

[0027] Further provided herein is a method of producing the monoclonal population of binding molecules disclosed herein, including culturing the host cell disclosed herein, and recovering the population of binding molecules.

[0028] ~~In another aspect, the present disclosure provides~~ ~~Also provided herein is~~ a method for producing a monoclonal population of highly sialylated multimeric binding molecules, including providing a cell line that expresses the monoclonal population of binding molecules, culturing the cell line, and recovering the monoclonal population of binding molecules, where each binding molecule includes ten or twelve IgM-derived heavy chains, where the IgM-derived heavy chains include glycosylated IgM heavy chain constant regions each associated with a binding domain that specifically binds to a target, where each IgM heavy chain constant region includes at least three, at least four, or at least five asparagine(N)-linked glycosylation motifs, where an N-linked glycosylation motif includes the amino acid sequence N-X₁-S/T, where N is asparagine, X₁ is any amino acid except proline, and S/T is serine or threonine, where 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 where the cell line, culture conditions, recovery process, or a combination thereof is optimized to enrich for complex glycans including at least one, two, three, or four sialic acid terminal monosaccharides per glycan.

[0029] Also provided herein is a method for producing a monoclonal population of highly sialylated multimeric binding molecules, including providing a cell line that expresses the monoclonal population of binding molecules, culturing the cell line, and recovering the monoclonal population of binding molecules, where each binding molecule includes ten or twelve IgM-derived heavy chains, where the IgM-derived heavy chains include glycosylated IgM heavy chain constant regions each associated with a binding domain that specifically binds to a target, where each IgM heavy chain constant region includes at least three, at least four, or at least five asparagine(N)-linked glycosylation motifs, where an N-linked glycosylation motif includes the amino acid sequence N-X₁-S/T, where N is asparagine, X₁ is any amino acid except proline, and S/T is serine or threonine, where 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 where the cell line, recovery process, or a combination thereof is optimized to enrich for complex glycans including at least one, two, three, or four sialic acid terminal monosaccharides per glycan.

[0030] In some [embodimentexamples](#), the cell line, culture conditions, recovery process, or a combination thereof is optimized to result in a monoclonal population of binding molecules including at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 124, at least 130, at least 140, or at least 146 moles sialic acid per mole of binding molecule. In some [embodimentexamples](#), the cell line, recovery process, or a combination thereof is optimized to result in a monoclonal population of binding molecules including at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, or at least 60 moles sialic acid per mole of binding molecule. In some [embodimentexamples](#), the cell line, recovery process, or a combination thereof is optimized to result in a monoclonal population of binding molecules including at least 30, at least 35, at least 40, at least 45, at least 50, or at least 60 moles sialic acid per mole of binding molecule. In some [embodimentexamples](#), the cell line, recovery process, or a combination thereof is optimized to result in a monoclonal population of binding molecules 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.

[0031] In some [embodimentexamples](#), the IgM heavy chain constant regions are derived from human IgM heavy chain constant regions including 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). In some [embodimentexamples](#), one, two, or all three of motifs N1, N2, and N3 in the population of binding molecules are occupied by complex glycans on average.

[0032] In some [embodimentexamples](#), the provided cell line is modified to overexpress a sialyltransferase. In some [embodimentexamples](#), the sialyltransferase includes human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1, SEQ ID NO: 3).

[0033] In some [embodimentexamples](#), the recovery process includes subjecting the monoclonal population of binding molecules to *in vitro* glycoengineering. In some [embodimentexamples](#), the *in vitro* glycoengineering includes contacting the monoclonal population of binding molecules with a soluble sialyltransferase and a sialic acid substrate. In some [embodimentexamples](#), the sialyltransferase includes a soluble variant of human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) (SEQ ID NO: 3). In some [embodimentexamples](#), the soluble variant of ST6GAL1 includes amino acids x to 406 of SEQ ID NO: 3, where x is an integer from 27 to 120. In some [embodimentexamples](#), the soluble variant of ST6GAL1 includes amino acids 120 to 406, 115 to 406, 110 to 406, 109 to 406, 105 to 406, 100 to 406, 95 to 406, 90 to 406, 89 to 406, 88 to 406, 87 to 406, 86 to 406, 85 to 406, 84 to 406, 83 to 406, 82 to 406, 81 to 406, 80 to 406, 75 to 406, 70 to 406, 65 to 406, 60 to 406, 55 to 406, 50 to 406, 45 to 406, 40 to 406, 35 to 406, 30 to 406, or 27 to 406 of SEQ ID NO: 3. In some [embodimentexamples](#), the sialic acid substrate includes cytidine monophosphate (CMP)-N-acetyl-neuraminic acid (CMP-NANA).

[0034] In some [embodimentexamples](#), the mass ratio of binding molecule: sialic acid substrate is about 1:4 to about 40:1. In some [embodimentexamples](#), the mass ratio of binding molecule: sialyltransferase is about 80:1 to about 10000:1. In some [embodimentexamples](#), the mass ratio of binding molecule: sialyltransferase is about 500:1. In some [embodimentexamples](#), the mass ratio of binding molecule: sialic acid substrate: sialyltransferase is about 500:62.5:1. In some [embodimentexamples](#), the mass ratio of binding molecule: sialyltransferase is about 2000:1. In some [embodimentexamples](#), the mass ratio of binding molecule: sialic acid substrate: sialyltransferase is about 2000:500:1. In some [embodimentexamples](#), the molar ratio of binding molecule: sialyltransferase is about 80:1. In some [embodimentexamples](#), the molar ratio of binding molecule: sialic acid substrate: sialyltransferase is about 80:500:1.

[0035] In some [embodimentexamples](#), the contacting of the monoclonal population of binding molecules with the soluble sialyltransferase and the sialic acid substrate comprises at least 30 minutes of contact. In some [embodimentexamples](#), the contacting comprises at least 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 24 hours, 36 hours, or 48 hours of contact. In some [embodimentexamples](#), the contacting of the monoclonal population of binding molecules with the soluble sialyltransferase and the sialic acid substrate occurs at about 2° C to about 40° C. In some [embodimentexamples](#), the contacting occurs at 15° C to about 37° C, 15° C to about 30° C, or 15° C to about 25° C.

[0036] In some [embodimentexamples](#), *in vitro* glycoengineering further includes contacting the monoclonal population of binding molecules with a galactosyltransferase and a galactose substrate. In some [embodimentexamples](#), the galactosyltransferase includes a soluble variant of human beta-1,4-galactosyltransferase 4 (B4GALT4) (SEQ ID NO: 4). In some [embodimentexamples](#), the galactose substrate includes uridine-diphosphate- α -D-galactose (UDP-Gal). In some [embodimentexamples](#), the contacting with the galactosyltransferase and a galactose substrate occurs prior to or simultaneously with the contacting with the soluble sialyltransferase and a sialic acid substrate.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0037] **FIG. 1A** shows the structure of a “simple” glycan. **FIG. 1B** shows an exemplary structure of an oligomannose glycan. **FIG. 1C** shows an exemplary structure of a complex glycan. **FIG. 1D** shows an exemplary structure of a hybrid glycan. Monosaccharides: darker circles = mannose; lighter circles = galactose; squares = N-acetylglucosamine; diamonds = N-acetylneuraminic acid (sialic acid or NANA); triangles = fucose. Derived from: Varki, A., and Schauer, R., *Essentials of Glycobiology*, 3d Edition, Chapter 8, Consortium of Glycobiology (2009).

[0038] **FIG. 2A** shows the structure of N-acetylneuraminic acid (sialic acid or NANA); **FIG. 2B** shows the structure of cytidine monophosphate N-acetylneuraminic acid (CMP-NANA).

[0039] **FIG. 3A** is a space-filling model of a human IgM heavy chain, showing the positions of the five N-linked glycosylation sites. **FIG. 3B** shows an alignment of the human IgM heavy chain constant region amino acid sequence (alleleIGHM*04, SEQ ID NO: 2) with those of mouse (GenBank: CAC20701.1, SEQ ID NO: 46) and cynomolgus monkey (amino acids 14 to 487 of GenBank: EHH62210.1, SEQ ID NO: 47). The amino acids corresponding to asparagine (N)-linked glycosylation motifs are boxed.

[0040] **FIG. 4** shows the amount of sialylation of anti-CD20 x CD3 IGM-A resulting from treatment with varying concentrations of truncated human α -2,6-sialyltransferase (ST6).

[0041] **FIG. 5** shows *in vitro* sialylation of two different IgM antibodies, anti-DR5 IgM-B and anti-DR5 IgM-C.

[0042] **FIG. 6** shows the pharmacokinetics of anti-CD20 x CD3 IGM-A and anti-CD20 x CD3 IGM-A-GEM antibodies in a mouse model.

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.~~

10.4. 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.

11.5. The monoclonal population of binding molecules of claim ~~410~~, wherein each binding molecule is pentameric and further comprises a J-chain, or functional fragment thereof, or a functional variant thereof.

12.6. The monoclonal population of binding molecules of claim ~~511~~, 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.

13.7. The monoclonal population of binding molecules of claim ~~115~~, 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.

14.8. The monoclonal population of binding molecules of claim ~~713~~, wherein the amino acid corresponding to Y102 of SEQ ID NO: 6 is substituted with alanine (A).

15.9. The monoclonal population of binding molecules of claim ~~814~~, wherein the J-chain comprises the amino acid sequence SEQ ID NO: 7.

16.10. The monoclonal population of binding molecules of claim ~~511~~, 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.

17.11. The monoclonal population of binding molecules of claim 1046, wherein the heterologous moiety is a polypeptide fused to the J-chain or fragment or variant thereof.

18.12. The monoclonal population of binding molecules of claim 1147, 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.~~

20.13. The monoclonal population of binding molecules of claim 1147, wherein the heterologous polypeptide comprises a scFv fragment.

21.14. The monoclonal population of binding molecules of claim 1320, wherein the heterologous scFv fragment binds to CD3ε.

22.15. A pharmaceutical composition comprising the monoclonal population of binding molecules of any one of claims 1 to 1421 and a pharmaceutically acceptable excipient.

23.16. A recombinant host cell that produces the monoclonal population of binding molecules of any one of claims 1 to 1421.

~~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.~~

25.17. 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.

~~26.18.~~ The method of claim ~~1725~~, 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.

~~27.19.~~ The method of claim ~~1725~~, 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).

~~28.20.~~ The method of claim ~~2517~~, wherein the provided cell line is modified to overexpress a sialyltransferase.

~~29.21.~~ The method of claim ~~2517~~, 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.

~~30.22.~~ The method of claim ~~2921~~, 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-acetylneuraminic acid (CMP-NANA).

~~31.23.~~ The method of claim ~~2921~~, 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.

~~32.24.~~ The method of any one of claims ~~29-21~~ to ~~3123~~, 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.