

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 comprises a pentameric IgM antibody comprising bivalent IgM binding units, wherein each binding unit comprises two IgM heavy chains each comprising a heavy chain variable domain (VH) situated amino terminal to a glycosylated IgM heavy chain constant region, and two immunoglobulin light chains each comprising a light chain variable domain (VL) situated amino terminal an IgM light chain constant region, and wherein the VH and VL combine to form an antigen-binding domain that specifically binds to a target, wherein each binding molecule further comprises a modified J-chain, a functional fragment thereof, or a functional variant thereof, comprising a heterologous polypeptide fused to the J-chain, functional fragment thereof, or functional variant thereof, wherein each glycosylated IgM heavy chain constant region comprises one, two, three, four, or five asparagine (N)-linked glycosylation motifs, wherein one or more of the N-linked glycosylation motifs 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 one, two, or three of the N-linked glycosylation motifs on each glycosylated IgM heavy chain constant region are occupied by a complex glycan, and wherein the monoclonal population of binding molecules comprises 35 to 65 moles sialic acid per mole of the binding molecules.

In another aspect, the present disclosure provides a method for producing a monoclonal population of 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 a pentameric or hexameric IgM antibody comprising bivalent IgM binding units, wherein each binding unit comprises two IgM heavy chains each comprising a heavy chain variable domain (VH) situated amino terminal to a glycosylated IgM heavy chain constant region, and two immunoglobulin light chains each comprising a light chain variable domain (VL) situated amino terminal to an IgM light chain constant region, and wherein the VH and VL combine to form an antigen-binding domain that specifically binds to a target, wherein each glycosylated IgM heavy chain constant region comprises three, four, or 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 one, two, or 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 one, two, three, or four sialic acid terminal monosaccharides per glycan, and wherein the monoclonal population of binding molecules comprises 35 to 65 moles sialic acid per mole of the binding molecules.

In another aspect, the present disclosure provides a method for producing a monoclonal population of sialylated multimeric binding molecules, comprising providing a cell line that is modified to overexpress a sialyltransferase and expresses the monoclonal population of binding molecules, culturing the cell line, and recovering the monoclonal population of binding molecules, wherein each binding molecule comprises a pentameric or hexameric IgM antibody comprising bivalent IgM binding units, wherein each binding unit comprises two IgM heavy chains each comprising a heavy chain variable domain (VH) situated amino terminal to a glycosylated IgM heavy chain constant region, and two immunoglobulin light chains each comprising a light chain variable domain (VL) situated amino terminal to an IgM light chain constant region, and wherein the VH and VL combine to form an antigen-binding domain that specifically binds to a target, wherein each glycosylated IgM heavy chain constant region comprises three, four, or 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 one, two, or 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 one, two, three, or four sialic acid terminal monosaccharides per glycan, and wherein the monoclonal population of binding molecules comprises 35 to 65 moles sialic acid per mole of the binding molecules.

[0008] In some examples, 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 examples, 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 examples, the monoclonal population of binding molecules includes about 40 to about 70, about 40 to about 60, about 40 to about

- [0025]** In another aspect, the present disclosure provides 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 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 example, the present disclosure provides 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

WHAT IS CLAIMED IS:

1. A monoclonal population of multimeric binding molecules, each binding molecule comprises a pentameric IgM antibody comprising bivalent IgM binding units, wherein each binding unit comprises two IgM heavy chains each comprising a heavy chain variable domain (VH) situated amino terminal to a glycosylated IgM heavy chain constant region, and two immunoglobulin light chains each comprising a light chain variable domain (VL) situated amino terminal an IgM light chain constant region, and wherein the VH and VL combine to form an antigen-binding domain that specifically binds to a target, wherein each binding molecule further comprises a modified J-chain, a functional fragment thereof, or a functional variant thereof, comprising a heterologous polypeptide fused to the J-chain, functional fragment thereof, or functional variant thereof, wherein each glycosylated IgM heavy chain constant region comprises one, two, three, four, or five asparagine (N)-linked glycosylation motifs, wherein one or more of the N-linked glycosylation motifs 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 one, two, or three of the N-linked glycosylation motifs on each glycosylated IgM heavy chain constant region are occupied by a complex glycan, and wherein the monoclonal population of binding molecules comprises 35 to 65 moles sialic acid per mole of the binding molecules.

2. The monoclonal population of binding molecules of claim 0, comprising 40 to 65, 40 to 55, 40 to 50, 50 to 65, or 60 to 65 moles sialic acid per mole of binding molecule.

3. The monoclonal population of binding molecules of claim 0, wherein one or more of the five N-linked glycosylation motifs N-X₁-S/T is at one or more of 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 any one of claims 1-3, 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.

5. The monoclonal population of binding molecules of claim 4, 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.

6. The monoclonal population of binding molecules of claim 5, wherein the amino acid corresponding to Y102 of SEQ ID NO: 6 is substituted with alanine (A).
7. The monoclonal population of binding molecules of claim 6, wherein the J-chain comprises the amino acid sequence SEQ ID NO: 7.
8. The monoclonal population of binding molecules of any one of claims 1-7, wherein the heterologous polypeptide is fused to the J-chain, functional fragment thereof, or functional variant thereof, via a peptide linker comprising at least 5 amino acids, but no more than 25 amino acids.
9. The monoclonal population of binding molecules of any one of claims 1-8, wherein the heterologous polypeptide comprises a scFv fragment.
10. The monoclonal population of binding molecules of claim 9, wherein the heterologous scFv fragment binds to CD3ε.
11. A pharmaceutical composition comprising the monoclonal population of binding molecules of any one of claims 0 to 10 and a pharmaceutically acceptable excipient.
12. A recombinant host cell that produces the monoclonal population of binding molecules of any one of claims 0 to 10.
13. A method for producing a monoclonal population of 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 a pentameric or hexameric IgM antibody comprising bivalent IgM binding units, wherein each binding unit comprises two IgM heavy chains each comprising a heavy chain variable domain (VH) situated amino terminal to a glycosylated IgM heavy chain constant region, and two immunoglobulin light chains each comprising a light chain variable domain (VL) situated amino terminal to an IgM light chain constant region, and wherein the VH and VL combine to form an antigen-binding domain that specifically binds to a target, wherein each glycosylated IgM heavy chain constant region comprises three, four, or 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 one, two, or 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 one, two, three, or four sialic acid terminal monosaccharides per glycan, and

wherein the monoclonal population of binding molecules comprises 35 to 65 moles sialic acid per mole of the binding molecules.

14. A method for producing a monoclonal population of sialylated multimeric binding molecules, comprising providing a cell line that is modified to overexpress a sialyltransferase and expresses the monoclonal population of binding molecules, culturing the cell line, and recovering the monoclonal population of binding molecules, wherein each binding molecule comprises a pentameric or hexameric IgM antibody comprising bivalent IgM binding units, wherein each binding unit comprises two IgM heavy chains each comprising a heavy chain variable domain (VH) situated amino terminal to a glycosylated IgM heavy chain constant region, and two immunoglobulin light chains each comprising a light chain variable domain (VL) situated amino terminal to an IgM light chain constant region, and wherein the VH and VL combine to form an antigen-binding domain that specifically binds to a target, wherein each glycosylated IgM heavy chain constant region comprises three, four, or 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 one, two, or 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 one, two, three, or four sialic acid terminal monosaccharides per glycan, and wherein the monoclonal population of binding molecules comprises 35 to 65 moles sialic acid per mole of the binding molecules.

15. The method of claim 13 or 14, wherein the cell line, recovery process, or a combination thereof is optimized to result in a monoclonal population of binding molecules comprising at least 35, at least 40, at least 45, at least 50, at least 60, or 65 moles sialic acid per mole of binding molecule; or comprising 40 to 65, 40 to 60, 40 to 55, 40 to 50, 50 to 65, or 60 to 65 moles sialic acid per mole of binding molecule.

16. The method of any one of claims 13 to 15, wherein one or more of the five N-linked glycosylation motifs N-X₁-S/T is at one or more 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).

17. The method of any one of claims 13 to 16, wherein the provided cell line is modified to overexpress a sialyltransferase.

18. The method of any one of claims 13 to 17, 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.

19. The method of claim 18, 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).

20. The method of claim 18 or 19, wherein the mass ratio of binding molecule: sialic acid substrate is 1:4 to 40:1 and/or the mass ratio of binding molecule: sialyltransferase is 80:1 to 5000:1.

21. The method of any one of claims 18 to 20, 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 2° C to 40° C.