(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 July 2021 (15.07.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/141902 A1

(51) International Patent Classification:
*A61K 39/395* (2006.01)

(21) International Application Number:
PCT/US2021/012192

(22) International Filing Date:
05 January 2021 (05.01.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/957,745 06 January 2020 (06.01.2020) US

(71) Applicant: **IGM BIOSCIENCES, INC.** [US/US]; 325 East Middlefield Road, Mountain View, California 94043 (US).

(72) Inventors: **KEYT, Bruce**; 1180 Lakeview Drive, Hillsborough, California 94010 (US). **BALIGA, Ramesh**; 2237 Hopkins Avenue, Redwood City, California 94062 (US). **RAHMAN, Sachi**; 4972 Hildasue Terrace, Fremont, California 94555 (US). **CARLIN, Kevin**; 20532 Deerpark Ct, Saratoga, California 95070 (US). **HINTON, Paul**; 321 Dunsmuir Ter. Unit 4, Sunnyvale, California 94085 (US). **SMITH, Mark**; 909 Alvarado Ave #11, Davis, California 95616 (US). **SAINI, Avneesh**; 466 Costa Mesa Terrace, Apt# C, Sunnyvale, California 94085 (US). **TRAN, Huyen**; 1399 Bouret Drive, San Jose, California 95118 (US). **PETERSON, Marvin**; 180 Terrazzo Circle, San Ramon, California 94583 (US).

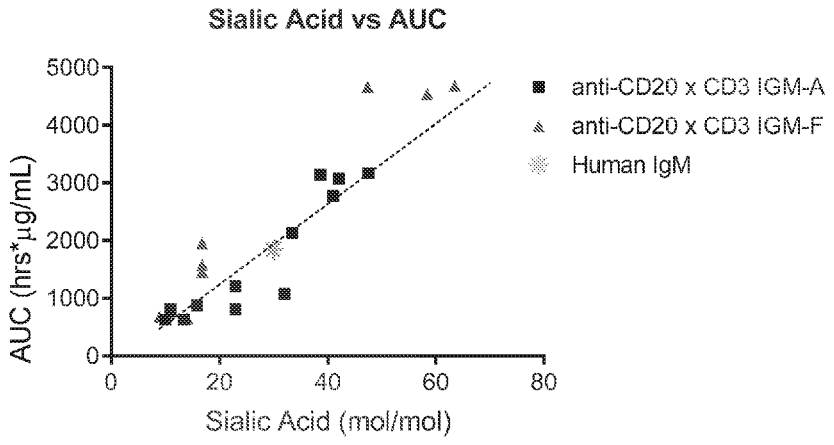
(74) Agent: **JARECKI, Jessica L.** et al.; IGM Biosciences, Inc., 325 East Middlefield Road, Mountain View, California 94043 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) **Title:** HIGHLY SIALYLATED MULTIMERIC BINDING MOLECULES

FIG. 15




(57) **Abstract:** This disclosure provides a monoclonal population of highly sialylated multimeric binding molecules where the population includes IgM antibodies, IgM-like antibodies, or other IgM-derived binding molecules, where the population of binding molecules has a higher level of sialic acid content than is found in normal serum IgM. Also provided are methods of producing such monoclonal populations of highly sialylated multimeric binding molecules.

WO 2021/141902 A1

*[Continued on next page]*

**Published:**

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

# HIGHLY SIALYLATED MULTIMERIC BINDING MOLECULES

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the benefit of U.S. Provisional Patent Application Serial No. 62/957,745, filed January 6, 2020, which is incorporated herein by reference in its entirety.

## SEQUENCE LISTING

**[0002]** The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The ASCII copy was created on January 5, 2021, is named 028WO1-Sequence-Listing, and is 92,335 bytes in size.

## BACKGROUND

**[0003]** Antibodies and antibody-like molecules that can multimerize, such as IgA and IgM antibodies, have emerged as promising drug candidates, *e.g.*, in the fields of immuno-oncology and infectious diseases, allowing for improved specificity, improved avidity, and the ability to bind to multiple binding targets. See, *e.g.*, U.S. Patent Nos. 9,951,134, 9,938,347, 10,351,631, 10,400,038, 10,570,191, 10,604,559, 10,618,978, 10,689,449, and 10,787,520, U.S. Patent Application Publication Nos. US 2019-0330374, US 2019-0330360, US 2019-0338040, US 2019-0338041, US 2019-0185570, and US 2019-0002566, US 2020-0239572, and PCT Publication Nos. WO 2018/187702 and WO 2019/165340, the contents of which are incorporated herein by reference in their entireties.

**[0004]** The pharmacokinetics (PK) and pharmacodynamics (PD) of multivalent antibodies are complex and depend on the structure of the monoclonal antibody both translationally and post-translationally, as well as the physiological system that it targets. Moreover, different antibody classes are typically processed within a subject via different cellular and physiological systems. For example, the IgG antibody class has a serum half-life of 20 days, whereas the half-lives for IgM and IgA antibodies are only about 5–8 days (Brekke, OH., and I. Sandlie, *Nature Reviews Drug Discovery 2*: 52-62 (2003)).

**[0005]** One of the key determinants of PK of an antibody or other biotherapeutic is its level and type of glycosylation (Higel, F. *et al. Eur. J. Pharm. Biopharm. 139*:123-131 (2019)).

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-$X_1$-S/T, where N is asparagine, $X_1$ 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.

**[0008]** In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the IgM heavy chain constant regions are human IgM heavy chain constant regions or variants thereof including five N-linked glycosylation motifs N-$X_1$-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 embodiments, motifs N1, N2, and N3 are occupied by complex glycans.

**[0010]** In some embodiments, 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 embodiments, 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 embodiments, the sialyltransferase includes human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) (SEQ ID NO: 3). In some embodiments, 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 embodiments, the galactosyltransferase includes human beta-1,4-galactosyltransferase 4 (B4GALT4) (SEQ ID NO: 4).

**[0012]** In some embodiments, the *in vitro* glycoengineering includes contacting the monoclonal population of binding molecules with a soluble sialyltransferase and a sialic acid substrate. In some embodiments, the sialyltransferase includes a soluble variant of human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) (SEQ ID NO: 3). In some embodiments, 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 embodiments, 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 embodiments, the sialic acid substrate includes cytidine monophosphate-N-acetyl-neuraminic acid (CMP-NANA).

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

**[0014]** In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the *in vitro* glycoengineering further includes contacting the monoclonal population of binding molecules with a galactosyltransferase and a galactose substrate. In some embodiments, the galactosyltransferase includes a soluble variant of human beta-1,4-galactosyltransferase 4 (B4GALT4) (SEQ ID NO: 4). In some embodiments, 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 embodiments, 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 embodiments, the galactose substrate includes uridine-diphosphate-α-D-galactose (UDP-Gal). In some embodiments, 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 embodiments, 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 embodiments, the binding domains associated with the IgM heavy chain constant regions of each binding molecule specifically bind to the same target. In some embodiments, the binding domains associated with the IgM heavy chain constant regions of each binding molecule are identical.

**[0017]** In some embodiments, the binding domains are antibody-derived antigen-binding domains. In some embodiments, 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 embodiments, each antigen-binding domain of each binding molecule binds to the same target. In some embodiments, each antigen-binding domain of each binding molecule is identical.

**[0018]** In some embodiments, the target is a target epitope, a target antigen, a target cell, a target organ, or a target virus.

**[0019]** In some embodiments, each binding molecule is pentameric and further includes a J-chain, or functional fragment thereof, or a functional variant thereof. In some embodiments, 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 embodiments, the J-chain includes an N-linked glycosylation motif N-$X_1$-S/T starting at amino acid positions corresponding to amino acid 49 of SEQ ID NO: 6 (motif N6).

**[0020]** In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the amino acid corresponding to Y102 of SEQ ID NO: 6 is substituted with alanine (A). In some embodiments, the J-chain includes the amino acid sequence SEQ ID NO: 7.

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

**[0023]** In some embodiments, 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 embodiments, 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 embodiments, the heterologous polypeptide includes a binding domain. In some embodiments, the binding domain of the heterologous polypeptide is an antibody or antigen-binding fragment thereof. In some embodiments, the antigen-binding fragment is a scFv fragment. In some embodiments, the heterologous scFv fragment binds to CD3ε. In some embodiments, 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-CD3ε 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 is a pharmaceutical composition comprising the monoclonal population of binding molecules disclosed herein and a pharmaceutically acceptable excipient.

**[0026]** 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]** 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-$X_1$-S/T, where N is asparagine, $X_1$ 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-$X_1$-S/T, where N is asparagine, $X_1$ 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the IgM heavy chain constant regions are derived from human IgM heavy chain constant regions including five N-linked glycosylation motifs N-$X_1$-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 embodiments, 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 embodiments, the provided cell line is modified to overexpress a sialyltransferase. In some embodiments, the sialyltransferase includes human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1, SEQ ID NO: 3).

**[0033]** In some embodiments, the recovery process includes subjecting the monoclonal population of binding molecules to *in vitro* glycoengineering. In some embodiments, the *in vitro* glycoengineering includes contacting the monoclonal population of binding molecules with a soluble sialyltransferase and a sialic acid substrate. In some embodiments, the sialyltransferase includes a soluble variant of human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) (SEQ ID NO: 3). In some embodiments, 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 embodiments, 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 embodiments, the sialic acid substrate includes cytidine monophosphate (CMP)-N-acetyl-neuraminic acid (CMP-NANA).

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

**[0035]** In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, *in vitro* glycoengineering further includes contacting the monoclonal population of binding molecules with a galactosyltransferase and a galactose substrate. In some embodiments, the galactosyltransferase includes a soluble variant of human beta-1,4-galactosyltransferase 4 (B4GALT4) (SEQ ID NO: 4). In some embodiments, the galactose substrate includes uridine-diphosphate-α-D-galactose (UDP-Gal). In some embodiments, 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 (allele IGHM*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.

[0043] **FIG. 7** shows SNA-I lectin labeling of subclones. Cells were labelled with SNA-1 lectin conjugated to fluorescein isothiocyanate (FITC). The geomean of the signal from 488em/530ex measured by a cytometer is shown for each subclone.

[0044] **FIG. 8A** shows a reduced, denatured BioRad® CriterionTGX Stain-Free Precast gel visualized and imaged according to manufacturer's instructions, for purified proteins from fermentations carried out on the 2,6-sialyl transferase pool as well as 2 subclones (25 and 47). **FIG. 8B** shows western blot on the same proteins in **FIG. 8A** using a biotinylated SNA-I lectin. A streptavidin horseradish peroxidase fusion was used for the blot.

[0045] **FIGS. 9A-9B** shows fermentation comparison data from a 3-L bioreactor for anti-CD20 x CD3 IGM-A producing cell lines. Two of the curves are shown for the control parental cell-line which does not have the 2,6-sialyltransferase gene and one for a production run with subclone 25 which has the 2,6-sialyltransferase gene. **FIG. 9A** shows the viable cell density (VCD) over the course of the run. **FIG. 9B** shows the viability of the cell-lines. **FIG. 9C** shows the titer as determined by size exclusion chromatography (SEC). **FIG. 9D** shows the ratio of the sialic acid measured on the purified IgM.

[0046] **FIG. 10A** shows a plot of the screening at the 96-well level for CHO cell clones transfected with 2,6-sialyltransferase. **FIG. 10B** shows a plot of the cytometry based analysis of cell surface 2,6-sialic acid levels.

[0047] **FIGS. 11A** and **11B** show the 2,3-sialic acid and 2,6-sialic acid levels for untransfected cells, respectively. **FIG. 11C** compares the 2,3-sialic acid and 2,6-sialic acid levels in untransfected and transfected cells.

[0048] **FIG. 12** shows T cell activation with various amounts of antibodies with a range of sialic acid levels.

[0049] **FIGS. 13A-13B** show a time course of sialylation of anti-CD20 x CD3 – IGM-A with various amounts of ST6 and CMP-NANA and at various temperatures.

[0050] **FIG. 14** shows a time course of sialylation of anti-CD20 x CD3 – IGM-A with various amounts of ST6 and CMP-NANA and room temperature.

[0051] **FIG. 15** shows a comparison of sialic acid levels and the resulting $AUC_{0-\infty}$ for various antibodies.

[0052] **FIG. 16** shows the pharmacokinetics of anti-CD20 x CD3 IGM-F (SA 18) and anti-CD20 x CD3 IGM-F-GEM (SA 51) antibodies in a cynomolgus monkey model.

[0053] **FIG. 17A** shows the relative numbers of cynomolgus B cells at each time point after administration of anti-CD20 x CD3 – IGM-F (SA 9 or 18) or anti-CD20 x CD3 – IGM-F-

GEM (SA 51). **FIG. 17B** shows the day that cynomolgus B cells began to recover after administration of anti-CD20 x CD3 – IGM-F (SA 9 or 18) or anti-CD20 x CD3 – IGM-F-GEM (SA 51).

## DETAILED DESCRIPTION

### Definitions

**[0054]** As used herein, the term "a" or "an" entity refers to one or more of that entity; for example, "a binding molecule," is understood to represent one or more binding molecules. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

**[0055]** Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

**[0056]** Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary of Biochemistry and Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

**[0057]** Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various embodiments or embodiments of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

**[0058]** As used herein, the term “polypeptide” is intended to encompass a singular “polypeptide” as well as plural “polypeptides,” and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids are included within the definition of "polypeptide,” and the term “polypeptide” can be used instead of any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, and derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide can be derived from a biological source or produced by recombinant technology but is not necessarily translated from a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis.

**[0059]** A polypeptide as disclosed herein can be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides can have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides which do not possess a defined three-dimensional structure, but rather can adopt many different conformations and are referred to as unfolded. As used herein, the term glycoprotein refers to a protein coupled to at least one carbohydrate moiety that is attached to the protein via an oxygen-containing or a nitrogen-containing side chain of an amino acid, *e.g.*, a serine or an asparagine. Asparagine (N)-linked glycans are described in more detail elsewhere in this disclosure.

**[0060]** By an "isolated" polypeptide or a fragment, variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated as disclosed herein, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

**[0061]** As used herein, the term "a non-naturally occurring polypeptide" or any grammatical variants thereof, is a conditional definition that explicitly excludes, but only excludes, those forms of the polypeptide that are, or might be, determined or interpreted by a judge or an administrative or judicial body, to be "naturally-occurring."

**[0062]** Other polypeptides disclosed herein are fragments, derivatives, analogs, or variants of the foregoing polypeptides, and any combination thereof. The terms "fragment," "variant," "derivative" and "analog" as disclosed herein include any polypeptides which retain at least some of the properties of the corresponding native antibody or polypeptide, for example, specifically binding to an antigen. Fragments of polypeptides include, for example, proteolytic fragments, as well as deletion fragments, in addition to specific antibody fragments discussed elsewhere herein. Variants of, *e.g.*, a polypeptide include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. In certain embodiments, variants can be non-naturally occurring. Non-naturally occurring variants can be produced using art-known mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions, or additions. Derivatives are polypeptides that have been altered so as to exhibit additional features not found on the original polypeptide. Examples include fusion proteins. As used herein a "derivative" of a polypeptide can also refer to a subject polypeptide having one or more amino acids chemically derivatized by reaction of a functional side group. Also included as "derivatives" are those polypeptides that contain one or more derivatives of the twenty standard amino acids. For example, 4-hydroxyproline can be substituted for proline; 5-hydroxylysine can be substituted for lysine; 3-methylhistidine can be substituted for histidine; homoserine can be substituted for serine; and ornithine can be substituted for lysine.

**[0063]** A "conservative amino acid substitution" is one in which one amino acid is replaced with another amino acid having a similar side chain. Families of amino acids having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). For example,

substitution of a phenylalanine for a tyrosine is a conservative substitution. In certain embodiments, conservative substitutions in the sequences of the polypeptides, binding molecules, and antibodies of the present disclosure do not abrogate the binding of the polypeptide, binding molecule, or antibody containing the amino acid sequence, to the antigen to which the antibody binds. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate antigen-binding are well-known in the art (see, *e.g.*, Brummell *et al.*, *Biochem.* 32: 1180-1 187 (1993); Kobayashi *et al.*, *Protein Eng.* 12(10):879-884 (1999); and Burks *et al.*, *Proc. Natl. Acad. Sci. USA* 94:.412-417 (1997)).

**[0064]** The term "polynucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids and refers to an isolated nucleic acid molecule or construct, *e.g.*, messenger RNA (mRNA), cDNA, or plasmid DNA (pDNA). A polynucleotide can comprise a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)). The terms "nucleic acid" or "nucleic acid sequence" refer to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide.

**[0065]** By an "isolated" nucleic acid or polynucleotide is intended any form of the nucleic acid or polynucleotide that is separated from its native environment. For example, gel-purified polynucleotide, or a recombinant polynucleotide encoding a polypeptide contained in a vector would be considered to be "isolated." Also, a polynucleotide segment, *e.g.*, a PCR product, which has been engineered to have restriction sites for cloning is considered to be "isolated." Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in a non-native solution such as a buffer or saline. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides, where the transcript is not one that would be found in nature. Isolated polynucleotides or nucleic acids further include such molecules produced synthetically. In addition, polynucleotide or a nucleic acid can be or can include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

**[0066]** As used herein, the term "a non-naturally occurring polynucleotide" or any grammatical variants thereof, is a conditional definition that explicitly excludes, but only excludes, those forms of the nucleic acid or polynucleotide that are, or might be,

determined or interpreted by a judge, or an administrative or judicial body, to be "naturally-occurring."

**[0067]** As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it can be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, *e.g.*, on a single vector, or in separate polynucleotide constructs, *e.g.*, on separate (different) vectors. Furthermore, any vector can contain a single coding region, or can comprise two or more coding regions, *e.g.*, a single vector can separately encode an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region. In addition, a vector, polynucleotide, or nucleic acid can include heterologous coding regions, either fused or unfused to another coding region. Heterologous coding regions include without limitation, those encoding specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

**[0068]** In certain embodiments, the polynucleotide or nucleic acid is DNA. In the case of DNA, a polynucleotide comprising a nucleic acid which encodes a polypeptide normally can include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. An operable association is when a coding region for a gene product, *e.g.*, a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter can be a cell-specific promoter that directs substantial transcription of the DNA in predetermined cells. Other transcription control elements, besides a promoter, for example

enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription.

**[0069]** A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions that function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (the immediate early promoter, in conjunction with intron-A), simian virus 40 (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit ß-globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (*e.g.*, promoters inducible by interferons or interleukins).

**[0070]** Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

**[0071]** In other embodiments, a polynucleotide can be RNA, for example, in the form of messenger RNA (mRNA), transfer RNA, or ribosomal RNA.

**[0072]** Polynucleotide and nucleic acid coding regions can be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide as disclosed herein. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells can have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the complete or "full length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, *e.g.*, an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, can be used. For example, the wild-type leader sequence can be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse ß-glucuronidase.

**[0073]** As used herein, the term "binding molecule" refers in its broadest sense to a molecule that specifically binds to a receptor or target, *e.g.*, an epitope or an antigenic determinant. As described further herein, a binding molecule can comprise one of more "binding domains," *e.g.*, "antigen-binding domains" described herein. A non-limiting example of a binding molecule is an antibody or antibody-like molecule as described in detail herein that retains antigen-specific binding. In certain embodiments a "binding molecule" comprises an antibody or antibody-like or antibody-derived molecule as described in detail herein.

**[0074]** As used herein, the terms "binding domain" or "antigen-binding domain" (can be used interchangeably) refer to a region of a binding molecule, *e.g.*, an antibody or antibody-like, or antibody-derived molecule, that is necessary and sufficient to specifically bind to a target, *e.g.*, an epitope, a polypeptide, a cell, or an organ. For example, an "Fv," *e.g.*, a heavy chain variable region and a light chain variable region of an antibody, either as two separate polypeptide subunits or as a single chain, is considered to be a "binding domain." Other antigen-binding domains include, without limitation, a single domain heavy chain variable region (VHH) of an antibody derived from a camelid species, or six immunoglobulin complementarity determining regions (CDRs) expressed in a fibronectin scaffold. A "binding molecule," or "antibody" as described herein can include one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or more "antigen-binding domains."

**[0075]** The terms "antibody" and "immunoglobulin" can be used interchangeably herein. An antibody (or a fragment, variant, or derivative thereof as disclosed herein, *e.g.*, an IgM-like antibody) includes at least the variable domain of a heavy chain (*e.g.*, from a camelid species) or at least the variable domains of a heavy chain and a light chain. Basic immunoglobulin structures in vertebrate systems are relatively well understood. *See*, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988). Unless otherwise stated, the term "antibody" encompasses anything ranging from a small antigen-binding fragment of an antibody to a full sized antibody, *e.g.*, an IgG antibody that includes two complete heavy chains and two complete light chains, an IgA antibody that includes four complete heavy chains and four complete light chains and includes a J-chain and/or a secretory component, or an IgM-derived binding molecule, *e.g.*, an IgM antibody or IgM-like antibody, that includes ten or twelve complete heavy

chains and ten or twelve complete light chains and optionally includes a J-chain or functional fragment or variant thereof.

**[0076]** The term "immunoglobulin" comprises various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon, (γ, μ, α, δ, ε) with some subclasses among them (*e.g.*, γ1-γ4 or α1-α2)). It is the nature of this chain that determines the "isotype" of the antibody as IgG, IgM, IgA IgD, or IgE, respectively. The immunoglobulin subclasses (subtypes) *e.g.*, $IgG_1$, $IgG_2$, $IgG_3$, $IgG_4$, $IgA_1$, $IgA_2$, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these immunoglobulins are readily discernible to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of this disclosure.

**[0077]** Light chains are classified as either kappa or lambda (κ, λ). Each heavy chain class can be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are expressed, *e.g.*, by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain. The basic structure of certain antibodies, *e.g.*, IgG antibodies, includes two heavy chain subunits and two light chain subunits covalently connected via disulfide bonds to form a "Y" structure, also referred to herein as an "H2L2" structure, or a "binding unit."

**[0078]** The term "binding unit" is used herein to refer to the portion of a binding molecule, *e.g.*, an antibody, antibody-like molecule, or antibody-derived molecule, antigen-binding fragment thereof, or multimerizing fragment thereof, which corresponds to a standard "H2L2" immunoglobulin structure, *i.e.*, two heavy chains or fragments thereof and two light chains or fragments thereof. In certain embodiments, *e.g.*, where the binding molecule is a bivalent IgG antibody or antigen-binding fragment thereof, the terms "binding molecule" and "binding unit" are equivalent. In other embodiments, *e.g.*, where the binding molecule is multimeric, *e.g.*, a dimeric IgA antibody or IgA-like antibody, a pentameric IgM antibody or IgM-like antibody, or a hexameric IgM antibody or IgM-like antibody, or any derivative thereof, the binding molecule comprises two or more "binding units." Two in the case of an IgA dimer, or five or six in the case of an IgM pentamer or hexamer, respectively. A binding unit need not include full-length antibody heavy and

light chains, but will typically be bivalent, *i.e.*, will include two "antigen-binding domains," as defined above. As used herein, certain binding molecules provided in this disclosure are "dimeric," and include two bivalent binding units that include IgA constant regions or multimerizing fragments thereof. Certain binding molecules provided in this disclosure are "pentameric" or "hexameric," and include five or six bivalent binding units that include IgM constant regions or multimerizing fragments or variants thereof. A binding molecule, *e.g.*, an antibody or antibody-like molecule or antibody-derived binding molecule, comprising two or more, *e.g.*, two, five, or six binding units, is referred to herein as "multimeric."

**[0079]** The term "J-chain" as used herein refers to the J-chain of IgM or IgA antibodies of any animal species, any functional fragment thereof, derivative thereof, and/or variant thereof, including a mature human J-chain, the amino acid sequence of which is presented as SEQ ID NO: 6. Various J-chain variants and modified J-chain derivatives are disclosed herein. As persons of ordinary skill in the art will recognize, "a functional fragment" or "a functional variant" includes those fragments and variants that can associate with IgM heavy chain constant regions to form a pentameric IgM antibody.

**[0080]** The term "modified J-chain" is used herein to refer to a derivative of a J-chain polypeptide comprising a heterologous moiety, *e.g.*, a heterologous polypeptide, *e.g.*, an extraneous binding domain or functional domain introduced into or attached to the J-chain sequence. The introduction can be achieved by any means, including direct or indirect fusion of the heterologous polypeptide or other moiety or by attachment through a peptide or chemical linker. The term "modified human J-chain" encompasses, without limitation, a native sequence human J-chain of the amino acid sequence of SEQ ID NO: 6 or functional fragment thereof, or functional variant thereof, modified by the introduction of a heterologous moiety, *e.g.*, a heterologous polypeptide, *e.g.*, an extraneous binding domain. In certain embodiments the heterologous moiety does not interfere with efficient polymerization of IgM into a pentamer or IgA into a dimer, and binding of such polymers to a target. Exemplary modified J-chains can be found, *e.g.*, in U.S. Patent Nos. 9,951,134, 10,400,038, and 10,618,978, and in U.S. Patent Application Publication No. US-2019-0185570, each of which is incorporated herein by reference in its entirety.

**[0081]** As used herein the term "IgM-derived binding molecule" refers collectively to native IgM antibodies, IgM-like antibodies, as well as other IgM-derived binding molecules comprising non-antibody binding and/or functional domains instead of an antibody

antigen binding domain or subunit thereof, and any fragments, *e.g.*, multimerizing fragments, variants, or derivatives thereof.

**[0082]** As used herein, the term "IgM-like antibody" refers generally to a variant antibody or antibody-derived binding molecule that still retains the ability to form hexamers or pentamers, *e.g.*, in association with a J-chain. An IgM-like antibody or other IgM-derived binding molecule typically includes at least the Cμ4-tp domains of the IgM constant region but can include heavy chain constant region domains from other antibody isotypes, *e.g.*, IgG, from the same species or from a different species. An IgM-like antibody or other IgM-derived binding molecule can likewise be an antibody fragment in which one or more constant regions are deleted, as long as the IgM-like antibody is capable of forming hexamers and/or pentamers. Thus, an IgM-like antibody or other IgM-derived binding molecule can be, *e.g.*, a hybrid IgM/IgG antibody or can be a "multimerizing fragment" of an IgM antibody.

**[0083]** The terms "valency," "bivalent," "multivalent" and grammatical equivalents, refer to the number of binding domains, *e.g.*, antigen-binding domains in given binding molecule, *e.g.*, antibody, antibody-derived, or antibody-like molecule, or in a given binding unit. As such, the terms "bivalent", "tetravalent", and "hexavalent" in reference to a given binding molecule, *e.g.*, an IgM antibody, IgM-like antibody, other IgM-derived binding molecule, or multimerizing fragment thereof, denote the presence of two antigen-binding domains, four antigen-binding domains, and six antigen-binding domains, respectively. A typical IgM antibody, IgM-like antibody, or other IgM-derived binding molecule, where each binding unit is bivalent, can have 10 or 12 valencies. A bivalent or multivalent binding molecule, *e.g.*, antibody or antibody-derived molecule, can be monospecific, *i.e.*, all of the antigen-binding domains are the same, or can be bispecific or multispecific, *e.g.*, where two or more antigen-binding domains are different, *e.g.*, bind to different epitopes on the same antigen, or bind to entirely different antigens.

**[0084]** The term "epitope" includes any molecular determinant capable of specific binding to an antigen-binding domain of an antibody, antibody-like, or antibody-derived molecule. In certain embodiments, an epitope can include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, can have three-dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of a target that is bound by an antigen-binding domain of an antibody.

**[0085]** The term "target" is used in the broadest sense to include substances that can be bound by a binding molecule, *e.g.*, antibody, antibody-like, or antibody-derived molecule. A target can be, *e.g.*, a polypeptide, a nucleic acid, a carbohydrate, a lipid, or other molecule, or a minimal epitope on such molecule. Moreover, a "target" can, for example, be a cell, an organ, or an organism, *e.g.*, an animal, plant, microbe, or virus, that comprises an epitope that can be bound by a binding molecule, *e.g.*, antibody, antibody-like, or antibody-derived molecule.

**[0086]** Both the light and heavy chains of antibodies, antibody-like, or antibody-derived molecules are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the variable light (VL) and variable heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant region domains of the light chain (CL) and the heavy chain (*e.g.*, CH1, CH2, CH3, or CH4) confer biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention, the numbering of the constant region domains increases as they become more distal from the antigen-binding site or amino-terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 (or CH4, *e.g.*, in the case of IgM) and CL domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

**[0087]** A "full length IgM antibody heavy chain" is a polypeptide that includes, in N-terminal to C-terminal direction, an antibody heavy chain variable domain (VH), an antibody heavy chain constant domain 1 (CM1 or Cμ1), an antibody heavy chain constant domain 2 (CM2 or Cμ2), an antibody heavy chain constant domain 3 (CM3 or Cμ3), and an antibody heavy chain constant domain 4 (CM4 or Cμ4) that can include a tailpiece.

**[0088]** As indicated above, variable region(s) allow a binding molecule, *e.g.*, antibody, antibody-like, or antibody-derived molecule, to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain, or subset of the complementarity determining regions (CDRs), of a binding molecule, *e.g.*, an antibody, antibody-like, or antibody-derived molecule, combine to form the antigen-binding domain. More specifically, an antigen-binding domain can be defined by three CDRs on each of the VH and VL chains. Certain antibodies form larger structures. For example, IgM can form a pentameric or hexameric molecule that includes five or six H2L2 binding units and optionally a J-chain covalently connected via disulfide bonds.

**[0089]** The six “complementarity determining regions” or “CDRs” present in an antibody antigen-binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen-binding domain as the antibody assumes its three-dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen-binding domain, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β-sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen-binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids that make up the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been defined in various different ways (*see*, "Sequences of Proteins of Immunological Interest," Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, *196*:901-917 (1987), which are incorporated herein by reference in their entireties).

**[0090]** In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described, for example, by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference. The Kabat and Chothia definitions include overlapping or subsets of amino acids when compared against each other. Nevertheless, application of either definition (or other definitions known to those of ordinary skill in the art) to refer to a CDR of an antibody or variant thereof is intended to be within the scope of the term as defined and used herein, unless otherwise indicated. The appropriate amino acids which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison. The exact amino acid numbers which encompass a particular

CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which amino acids comprise a particular CDR given the variable region amino acid sequence of the antibody.

Table 1 CDR Definitions*

| | Kabat | Chothia |
|---|---|---|
| VH CDR1 | 31-35 | 26-32 |
| VH CDR2 | 50-65 | 52-58 |
| VH CDR3 | 95-102 | 95-102 |
| VL CDR1 | 24-34 | 26-32 |
| VL CDR2 | 50-56 | 50-52 |
| VL CDR3 | 89-97 | 91-96 |

*Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat *et al.* (see below).

**[0091]** Antibody variable domains can also be analyzed, *e.g.*, using the IMGT information system (imgt_dot_cines_dot_fr/) (IMGT®/V-Quest) to identify variable region segments, including CDRs. (See, *e.g.*, Brochet *et al.*, *Nucl. Acids Res. 36*:W503-508, 2008).

**[0092]** Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless use of the Kabat numbering system is explicitly noted, however, consecutive numbering is used for all amino acid sequences in this disclosure.

**[0093]** The Kabat numbering system for the human IgM constant domain can be found in Kabat, et. al. "Tabulation and Analysis of Amino acid and nucleic acid Sequences of Precursors, V-Regions, C-Regions, J-Chain, T-Cell Receptors for Antigen, T-Cell Surface Antigens, β-2 Microglobulins, Major Histocompatibility Antigens, Thy-1, Complement, C-Reactive Protein, Thymopoietin, Integrins, Post-gamma Globulin, α-2 Macroglobulins, and Other Related Proteins," U.S. Dept. of Health and Human Services (1991). IgM constant regions can be numbered sequentially (*i.e.*, amino acid #1 starting with the first amino acid of the constant region, or by using the Kabat numbering scheme. A comparison of the numbering of two alleles of the human IgM constant region sequentially (presented

herein as SEQ ID NO: 1 (allele IGHM*03) and SEQ ID NO: 2 (allele IGHM*04)) and by the Kabat system is set out below. The underlined amino acid residues are not accounted for in the Kabat system ("X," double underlined below, can be serine (S) (SEQ ID NO: 1) or glycine (G) (SEQ ID NO: 2)):

Sequential (SEQ ID NO: 1 or SEQ ID NO: 2)/KABAT numbering key for IgM heavy chain

```
  1/127 GSASAPTLFP LVSCENSPSD TSSVAVGCLA QDFLPDSITF SWKYKNNSDI

 51/176 SSTRGFPSVL RGGKYAATSQ VLLPSKDVMQ GTDEHVVCKV QHPNGNKEKN

101/226 VPLPVIAELP PKVSVFVPPR DGFFGNPRKS KLICQATGFS PRQIQVSWLR

151/274 EGKQVGSGVT TDQVQAEAKE SGPTTYKVTS TLTIKESDWL XQSMFTCRVD

201/324 HRGLTFQQNA SSMCVPDQDT AIRVFAIPPS FASIFLTKST KLTCLVTDLT

251/374 TYDSVTISWT RQNGEAVKTH TNISESHPNA TFSAVGEASI CEDDWNSGER

301/424 FTCTVTHTDL PSPLKQTISR PKGVALHRPD VYLLPPAREQ LNLRESATIT

351/474 CLVTGFSPAD VFVQWMQRGQ PLSPEKYVTS APMPEPQAPG RYFAHSILTV

401/524 SEEEWNTGET YTCVVAHEAL PNRVTERTVD KSTGKPTLYN VSLVMSDTAG

451/574 TCY
```

**[0094]** Binding molecules, *e.g.*, antibodies, antibody-like, or antibody-derived molecules, antigen-binding fragments, variants, or derivatives thereof, and/or multimerizing fragments thereof include, but are not limited to, polyclonal, monoclonal, human, humanized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and $F(ab')_2$, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VL or VH domain, fragments produced by a Fab expression library. ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019.

**[0095]** By "specifically binds," it is generally meant that a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, a binding molecule, *e.g.*, antibody, antibody-like, or antibody-derived molecule, is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify

the relative affinity by which a certain binding molecule binds to a certain epitope. For example, binding molecule "A" can be deemed to have a higher specificity for a given epitope than binding molecule "B," or binding molecule "A" can be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

**[0096]** A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof disclosed herein can be said to bind a target antigen with an off rate (k(off)) of less than or equal to 5 X $10^{-2}$ $sec^{-1}$, $10^{-2}$ $sec^{-1}$, 5 X $10^{-3}$ $sec^{-1}$, $10^{-3}$ $sec^{-1}$, 5 X $10^{-4}$ $sec^{-1}$, $10^{-4}$ $sec^{-1}$, 5 X $10^{-5}$ $sec^{-1}$, or $10^{-5}$ $sec^{-1}$ 5 X $10^{-6}$ $sec^{-1}$, $10^{-6}$ $sec^{-1}$, 5 X $10^{-7}$ $sec^{-1}$ or $10^{-7}$ $sec^{-1}$.

**[0097]** A binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative disclosed herein can be said to bind a target antigen with an on rate (k(on)) of greater than or equal to $10^{3}$ $M^{-1}$ $sec^{-1}$, 5 X $10^{3}$ $M^{-1}$ $sec^{-1}$, $10^{4}$ $M^{-1}$ $sec^{-1}$, 5 X $10^{4}$ $M^{-1}$ $sec^{-1}$, $10^{5}$ $M^{-1}$ $sec^{-1}$, 5 X $10^{5}$ $M^{-1}$ $sec^{-1}$, $10^{6}$ $M^{-1}$ $sec^{-1}$, or 5 X $10^{6}$ $M^{-1}$ $sec^{-1}$ or $10^{7}$ $M^{-1}$ $sec^{-1}$.

**[0098]** A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof is said to competitively inhibit binding of a reference antibody or antigen-binding fragment to a given epitope if it preferentially binds to that epitope to the extent that it blocks, to some degree, binding of the reference antibody or antigen-binding fragment to the epitope. Competitive inhibition can be determined by any method known in the art, for example, competition ELISA assays. A binding molecule can be said to competitively inhibit binding of the reference antibody or antigen-binding fragment to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

**[0099]** As used herein, the term "affinity" refers to a measure of the strength of the binding of an individual epitope with one or more antigen-binding domains, *e.g.*, of an immunoglobulin molecule. *See*, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) at pages 27-28. As used herein, the term "avidity" refers to the overall stability of the complex between a population of antigen-binding domains and an antigen. *See*, *e.g.*, Harlow at pages 29-34. Avidity is related to both the affinity of individual antigen-binding domains in the population with specific epitopes, and also the valencies of the immunoglobulins and the antigen. For example, the interaction between a bivalent monoclonal antibody and an antigen with a highly repeating epitope structure, such as a polymer, would be one of high avidity. An interaction between a bivalent monoclonal antibody with a receptor present at a high density on a cell surface would also be of high avidity.

**[0100]** Binding molecules, *e.g.*, antibodies or fragments, variants, or derivatives thereof as disclosed herein can also be described or specified in terms of their cross-reactivity. As used herein, the term "cross-reactivity" refers to the ability of a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof, specific for one antigen, to react with a second antigen; a measure of relatedness between two different antigenic substances. Thus, a binding molecule is cross reactive if it binds to an epitope other than the one that induced its formation. The cross-reactive epitope generally contains many of the same complementary structural features as the inducing epitope, and in some cases, can actually fit better than the original.

**[0101]** A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can also be described or specified in terms of their binding affinity to an antigen. For example, a binding molecule can bind to an antigen with a dissociation constant or $K_D$ no greater than $5 \times 10^{-2}$ M, $10^{-2}$ M, $5 \times 10^{-3}$ M, $10^{-3}$ M, $5 \times 10^{-4}$ M, $10^{-4}$ M, $5 \times 10^{-5}$ M, $10^{-5}$ M, $5 \times 10^{-6}$ M, $10^{-6}$ M, $5 \times 10^{-7}$ M, $10^{-7}$ M, $5 \times 10^{-8}$ M, $10^{-8}$ M, $5 \times 10^{-9}$ M, $10^{-9}$ M, $5 \times 10^{-10}$ M, $10^{-10}$ M, $5 \times 10^{-11}$ M, $10^{-11}$ M, $5 \times 10^{-12}$ M, $10^{-12}$ M, $5 \times 10^{-13}$ M, $10^{-13}$ M, $5 \times 10^{-14}$ M, $10^{-14}$ M, $5 \times 10^{-15}$ M, or $10^{-15}$ M.

**[0102]** "Antigen-binding antibody fragments" including single-chain antibodies or other antigen-binding domains can exist alone or in combination with one or more of the following: hinge region, CH1, CH2, CH3, or CH4 domains, J-chain, or secretory component. Also included are antigen-binding fragments that can include any combination of variable region(s) with one or more of a hinge region, CH1, CH2, CH3, or CH4 domains, a J-chain, or a secretory component. Binding molecules, *e.g.*, antibodies, or antigen-binding fragments thereof can be from any animal origin including birds and mammals. The antibodies can be, *e.g.*, human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region can be condricthoid in origin (*e.g.*, from sharks). As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and can in some instances express endogenous immunoglobulins and some not, as described *infra* and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati *et al*. According to embodiments of the present disclosure, an IgM antibody, IgM-like antibody, or other IgM-derived binding molecule as provided herein can include an antigen-binding fragment of an antibody, *e.g.*, a scFv fragment, so

long as the IgM antibody, IgM-like antibody, or other IgM-derived binding molecule is able to form a multimer, *e.g.*, a hexamer or a pentamer. As used herein such a fragment comprises a "multimerizing fragment."

**[0103]** As used herein, the term "heavy chain subunit" includes amino acid sequences derived from an immunoglobulin heavy chain, a binding molecule, *e.g.*, an antibody, antibody-like, or antibody-derived molecule comprising a heavy chain subunit can include at least one of: a VH domain, a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, a CH4 domain, or a variant or fragment thereof. For example, a binding molecule, *e.g.*, an antibody, antibody-like, or antibody-derived molecule, or fragment, *e.g.*, multimerizing fragment, variant, or derivative thereof can include without limitation, in addition to a VH domain: a CH1 domain; a CH1 domain, a hinge, and a CH2 domain; a CH1 domain and a CH3 domain; a CH1 domain, a hinge, and a CH3 domain; or a CH1 domain, a hinge domain, a CH2 domain, and a CH3 domain. In certain embodiments a binding molecule, *e.g.*, an antibody, antibody-like, or antibody-derived molecule, or fragment, *e.g.*, multimerizing fragment, variant, or derivative thereof can include, in addition to a VH domain, a CH3 domain and a CH4 domain; or a CH3 domain, a CH4 domain, and a J-chain. Further, a binding molecule, *e.g.*, an antibody, antibody-like, or antibody-derived molecule, for use in the disclosure can lack certain constant region portions, *e.g.*, all or part of a CH2 domain. It will be understood by one of ordinary skill in the art that these domains (*e.g.*, the heavy chain subunit) can be modified such that they vary in amino acid sequence from the original immunoglobulin molecule. According to embodiments of the present disclosure, an IgM antibody, IgM-like antibody, or other IgM-derived binding molecule as provided herein comprises sufficient portions of an IgM heavy chain constant region to allow the IgM antibody, IgM-like antibody, or other IgM-derived binding molecule to form a multimer, *e.g.*, a hexamer or a pentamer. As used herein such a fragment comprises a "multimerizing fragment."

**[0104]** As used herein, the term "light chain subunit" includes amino acid sequences derived from an immunoglobulin light chain. The light chain subunit includes at least a VL, and can further include a CL (*e.g.*, Cκ or Cλ) domain.

**[0105]** Binding molecules, *e.g.*, antibodies, antibody-like molecules, antibody-derived molecules, antigen-binding fragments, variants, or derivatives thereof, or multimerizing fragments thereof can be described or specified in terms of the epitope(s) or portion(s) of

a target, *e.g.*, a target antigen that they recognize or specifically bind. The portion of a target antigen that specifically interacts with the antigen-binding domain of an antibody is an "epitope," or an "antigenic determinant." A target antigen can comprise a single epitope or at least two epitopes, and can include any number of epitopes, depending on the size, conformation, and type of antigen.

**[0106]** As used herein the term "disulfide bond" includes the covalent bond formed between two sulfur atoms, *e.g.*, in cysteine residues of a polypeptide. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. Disulfide bonds can be "intra-chain," *i.e.*, linking to cysteine residues in a single polypeptide or polypeptide subunit, or can be "inter-chain," *i.e.*, linking two separate polypeptide subunits, *e.g.*, an antibody heavy chain and an antibody light chain, to antibody heavy chains, or an IgM or IgA antibody heavy chain constant region and a J-chain.

**[0107]** As used herein, the term "chimeric antibody" refers to an antibody in which the immunoreactive region or site is obtained or derived from a first species and the constant region (which can be intact, partial, or modified) is obtained from a second species. In some embodiments the target binding region or site will be from a non-human source (*e.g.*, mouse or primate) and the constant region is human.

**[0108]** The terms "multispecific antibody" or "bispecific antibody" refer to an antibody, antibody-like, or antibody-derived molecule that has antigen-binding domains for two or more different epitopes within a single antibody molecule. Other binding molecules in addition to the canonical antibody structure can be constructed with two binding specificities. Epitope binding by bispecific or multispecific antibodies can be simultaneous or sequential. Triomas and hybrid hybridomas are two examples of cell lines that can secrete bispecific antibodies. Bispecific antibodies can also be constructed by recombinant means. (Ströhlein and Heiss, *Future Oncol. 6*:1387-94 (2010); Mabry and Snavely, *IDrugs. 13*:543-9 (2010)). A bispecific antibody can also be a diabody.

**[0109]** As used herein, the term "engineered antibody" refers to an antibody in which a variable domain, constant region, and/or J-chain is altered by at least partial replacement of one or more amino acids. In certain embodiments entire CDRs from an antibody of known specificity can be grafted into the framework regions of a heterologous antibody. Although alternate CDRs can be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, CDRs can also be

derived from an antibody of different class, *e.g.*, from an antibody from a different species. An engineered antibody in which one or more "donor" CDRs from a non-human antibody of known specificity are grafted into a human heavy or light chain framework region is referred to herein as a "humanized antibody." In certain embodiments not all of the CDRs are replaced with the complete CDRs from the donor variable region and yet the antigen-binding capacity of the donor can still be transferred to the recipient variable domains. Given the explanations set forth in, *e.g.*, U. S. Pat. Nos. 5,585,089, 5,693,761, 5,693,762, and 6,180,370, it will be well within the competence of those skilled in the art, either by carrying out routine experimentation or by trial and error testing, to obtain a functional engineered or humanized antibody.

**[0110]** As used herein the term “engineered” includes manipulation of nucleic acid or polypeptide molecules by synthetic means (*e.g.*, by recombinant techniques, *in vitro* peptide synthesis, by enzymatic or chemical coupling of peptides, nucleic acids, or glycans, or some combination of these techniques).

**[0111]** As used herein, the terms "linked," "fused" or "fusion" or other grammatical equivalents can be used interchangeably. These terms refer to the joining together of two more elements or components, by whatever means including chemical conjugation or recombinant means. An "in-frame fusion" refers to the joining of two or more polynucleotide open reading frames (ORFs) to form a continuous longer ORF, in a manner that maintains the translational reading frame of the original ORFs. Thus, a recombinant fusion protein is a single protein containing two or more segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally so joined in nature.) Although the reading frame is thus made continuous throughout the fused segments, the segments can be physically or spatially separated by, for example, in-frame linker sequence. For example, polynucleotides encoding the CDRs of an immunoglobulin variable region can be fused, in-frame, but be separated by a polynucleotide encoding at least one immunoglobulin framework region or additional CDR regions, as long as the "fused" CDRs are co-translated as part of a continuous polypeptide.

**[0112]** In the context of polypeptides, a "linear sequence" or a "sequence" is an order of amino acids in a polypeptide in an amino to carboxyl terminal direction in which amino acids that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide. A portion of a polypeptide that is “amino-terminal” or “N-terminal” to another portion of a polypeptide is that portion that comes earlier in the sequential

polypeptide chain. Similarly, a portion of a polypeptide that is "carboxy-terminal" or "C-terminal" to another portion of a polypeptide is that portion that comes later in the sequential polypeptide chain. For example, in a typical antibody, the variable domain is "N-terminal" to the constant region, and the constant region is "C-terminal" to the variable domain.

**[0113]** The term "expression" as used herein refers to a process by which a gene produces a biochemical, for example, a polypeptide. The process includes any manifestation of the functional presence of the gene within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression. It includes without limitation transcription of the gene into RNA, *e.g.*, messenger RNA (mRNA), and the translation of such mRNA into polypeptide(s). If the final desired product is a biochemical, expression includes the creation of that biochemical and any precursors. Expression of a gene produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.*, a messenger RNA produced by transcription of a gene, or a polypeptide that is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation, or polypeptides with post translational modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, proteolytic cleavage, and the like.

**[0114]** The terms "N-linked oligosaccharide" "N-linked sugar," "N-linked glycan" or other similar or grammatical variants, denote oligosaccharide chains that are linked to a peptide backbone via an asparagine residue. All N-linked oligosaccharides have a common pentasaccharide core of Man3GlcNAc2, also called a "simple oligosaccharide," see **FIG. 1A**. N-linked glycans can be generally classified into three types: (1) oligomannose, in which only mannose residues are attached to the core (**FIG. 1B**); (2) complex, in which "antennae" initiated by N-acetylglucosaminyltransferases (GlcNAcTs) are attached to the core (**FIG. 1C**); and (3) hybrid, in which only mannose residues are attached to the Manα1–6 arm of the core and one or two antennae are on the Manα1–3 arm (**FIG. 1D**). See, *e.g.*, Varki, A., and Schauer, R., Essentials of Glycobiology, 3d Edition, Chapter 8, Consortium of Glycobiology (2009).

**[0115]** The term "glycosyltransferase" denotes an enzyme capable of transferring a monosaccharide moiety from a nucleotide sugar to an acceptor molecule such as an oligosaccharide. Examples of such glycosyltransferases include, but not limited to glucosyltransferases, mannosyltransferases, galactosyltransferases, and sialyltransferases.

These enzymes are typically type II membrane proteins residing in the Golgi apparatus of cells, with the active portion of the enzyme residing in the Golgi lumen. In glycosyltransferase catalysis, the monosaccharide substrate units glucose (Glc), galactose (Gal), N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), glucuronic acid (GlcUA), galacturonic acid (GalUA) and xylose are activated as uridine diphosphate (UDP)-α-D derivatives; arabinose is activated as a UDP-β-L derivative; mannose (Man) and fucose are activated as GDP-α-D and GDP-β-L derivatives, respectively; and sialic acid (=β-D-Neu5Ac; =Neu5Ac; =SA; =NANA) is activated as a CMP derivative of sialic acid. See, *e.g.*, U.S. Patent Application Publication No. US 2017/0298405.

**[0116]** The term "sialic acid" denotes any member of a family of nine-carbon carboxylated sugars. The most common member of the sialic acid family is N-acetyl-neuraminic acid (2-keto-5-acetamido-3,5-dideoxy-D-glycero-D-galactononulopyranos-l-onic acid (often abbreviated as Neu5Ac, NeuAc, or NANA). **FIG. 2A**, see, *e.g.*, Varki, A., and Schauer, R., Essentials of Glycobiology, 3d Edition, Chapter 14, Consortium of Glycobiology (2009).

**[0117]** A sialyltransferase (="ST") is a glycosyltransferase that catalyzes transfer of a sialic acid residue from a donor substrate to, *e.g.*, a terminal monosaccharide acceptor group of an N-linked glycan of a glycoprotein. Mammalian sialyltransferases, including human ST species, use a common donor substrate which is cytidine-5′-monophospho-N-acetylneuraminic acid (=CMP-β-D-Neu5Ac; =CMP-Neu5Ac; =CMP-NANA; =CMP-sialic acid; =CMP-SA, **FIG. 2B**). Other functional equivalents are known, including, but not limited to azido-CMP-sialic acid to be used for glycan labelling by "click" chemistry. See, *e.g.*, Moh, *et al.*, *Anal. Biochem. 584*:11385 (2019). Transfer and covalent coupling of a sialic acid residue (or the functional equivalent thereof) to a receptor site is also referred to as "sialylating" and "sialylation."

**[0118]** A terminal sialic acid residue can be coupled to a galactose residue by various linkages, *e.g.*, (i) α2→3 (α2,3) linked to a galactose or (ii) α2→6 (α2,6) linked to a galactose. A sialyltransferase enzyme is generally named and classified according to its respective monosaccharide acceptor substrate and according to the position of the glycosidic bond it catalyzes. Exemplary eukaryotic sialyltransferases include (i) ST3Gal (found, *e.g.*, in CHO cells) and (ii) ST6Gal found in human cells. Shorthand reference to "ST3" specifically encompasses the sialyltransferases catalyzing an α2,3 sialylation.

Shorthand reference to "ST6" specifically encompasses the sialyltransferases catalyzing an α2,6 sialylation.

**[0119]** The disaccharide moiety β-D-galactosyl-1,4-N-acetyl-β-D-glucosamine (=Galβ1,4GlcNAc) is a frequent sialic acid acceptor of the antennae of N-linked glycans of glycoproteins. In addition, a terminal Galβ1,4GlcNAc moiety can be generated in certain target glycoproteins as a result of galactosyltransferase enzymatic activity, *e.g.*, human β-1,4-galactosyltransferase 4 (="hB4GALT4"). The enzyme β-galactoside-α2,6-sialyltransferase (="ST6Gal") is able to catalyze α2,6-sialylation of a terminal Galβ1,4GlcNAc acceptor moiety of a glycan or a branch of a glycan, or antenna.

**[0120]** The activity of ST6Gal enzymes catalyzes transfer of a Neu5Ac residue to the C6 hydroxyl group of a free galactosyl residue being part of terminal Galβ1,4GlcNAc in a glycan or an antenna of a glycan, thereby forming in the glycan a terminal sialic acid residue α2→6 linked to the galactosyl residue of the Galβ1,4GlcNAc moiety.

**[0121]** The wild-type polypeptide of human β-galactoside-α-2,6-sialyltransferase I (hST6Gal-I, UniProtKB/Swiss-Prot: P15907.1), is presented as SEQ ID NO: 3. Mammalian sialyltransferases share with other mammalian Golgi-resident glycosyltransferases a type II architecture with a cytoplasmic N-terminal tail, a transmembrane region, a stem region of variable length, and a C-terminal catalytic domain in the lumen of the Golgi apparatus. The cytoplasmic region of hST6GAL-1 includes amino acids 1-9 of SEQ ID NO: 3, the transmembrane region includes amino acids 10-26 of SEQ ID NO: 3, and the luminal region includes amino acids 27-406 of SEQ ID NO: 3. A soluble variant of hST6Gal-I would lack at least the transmembrane region and could further lack the N-terminal cytoplasmic region, and some portion of the luminal region, provided that the enzyme retains catalytic activity. In certain embodiments, a soluble variant of ST6GAL1 can include amino acids x to 406 of SEQ ID NO: 3, where x is an integer from 27 to 120. For example, a soluble variant of ST6GAL1 can include 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. US Patent Application No. US 2017/0298405 reports that amino acids 90-109 of SEQ ID NO: 3 confers an additional sialidase activity to the enzyme in the presence of free CMP.

**[0122]** The wild-type polypeptide of human β-1,4-galactosyltransferase 4 (hB4GALT4, UniProtKB/Swiss-Prot: O60513.1) is presented as SEQ ID NO: 4. This enzyme likewise has a type II architecture with a cytoplasmic N-terminal region, a transmembrane region, a stem region of variable length, and a C-terminal catalytic region in the lumen of the Golgi apparatus. The cytoplasmic region of hB4GALT4 includes amino acids 1-12 of SEQ ID NO: 4, the transmembrane region includes amino acids 13-38 of SEQ ID NO: 4, and the luminal region includes amino acids 39-344 of SEQ ID NO: 4. A soluble variant of hB4GALT4 would lack at least the transmembrane region and could further lack the N-terminal cytoplasmic region, and some portion of the luminal region, provided that the enzyme retains catalytic activity. In certain embodiments, a soluble variant of hB4GALT4 can include amino acids x to 344 of SEQ ID NO: 4, wherein x is an integer from 39 to 120. For example, a soluble variant of hB4GALT4 can include 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.

**[0123]** Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt or slow the progression of an existing diagnosed pathologic condition or disorder. Terms such as “prevent,” “prevention,” “avoid,” “deterrence” and the like refer to prophylactic or preventative measures that prevent the development of an undiagnosed targeted pathologic condition or disorder. Thus, “those in need of treatment” can include those already with the disorder and/or those prone to have the disorder.

**[0124]** As used herein the terms “serum half-life” or “plasma half-life” refer to the time it takes (*e.g.*, in minutes, hours, or days) following administration for the serum or plasma concentration of a drug, *e.g.*, a binding molecule such as an antibody, antibody-like, or antibody-derived molecule or fragment, *e.g.*, multimerizing fragment thereof as described herein, to be reduced by 50%. Two half-lives can be described: the alpha half-life, α half-life, or $t_{1/2}\alpha$, which is the rate of decline in plasma concentrations due to the process of drug redistribution from the central compartment, *e.g.*, the blood in the case of intravenous delivery, to a peripheral compartment (*e.g.*, a tissue or organ), and the beta half-life, β half-life, or $t_{1/2}\beta$ which is the rate of decline due to the processes of excretion or metabolism.

**[0125]** As used herein the term “area under the plasma drug concentration-time curve” or “AUC” reflects the actual body exposure to drug after administration of a dose of the drug

and is expressed in mg*h/L. This area under the curve can be measured, *e.g.*, from time 0 ($t_0$) to infinity ($\infty$) and is dependent on the rate of elimination of the drug from the body and the dose administered.

**[0126]** As used herein, the term "mean residence time" or "MRT" refers to the average length of time the drug remains in the body.

**[0127]** By "subject" or "individual" or "animal" or "patient" or "mammal," is meant any subject. In certain embodiments the subject is a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, swine, cows, bears, and so on.

**[0128]** As used herein, as the term "a subject that would benefit from therapy" refers to a subset of subjects, from amongst all prospective subjects, which would benefit from administration of a given therapeutic agent, *e.g.*, a binding molecule such as an antibody, comprising one or more antigen-binding domains. Such binding molecules, *e.g.*, antibodies, can be used, *e.g.*, for a diagnostic procedure and/or for treatment or prevention of a disease.

IgM antibodies, IgM-like antibodies, other IgM-derived binding molecules, and populations of such molecules

**[0129]** IgM is the first immunoglobulin produced by B cells in response to stimulation by antigen. Naturally-occurring IgM is naturally present at around 1.5 mg/ml in serum with a half-life of about 5 days. IgM is a pentameric or hexameric molecule and thus includes five or six binding units. An IgM binding unit typically includes two light and two heavy chains. While an IgG heavy chain constant region contains three heavy chain constant domains (CH1, CH2 and CH3), the heavy (μ) constant region of IgM additionally contains a fourth constant domain (CH4) and includes a C-terminal "tailpiece." The human IgM constant region typically comprises the amino acid sequence SEQ ID NO: 1 (identical to, *e.g.*, GenBank Accession Nos. pir||S37768, CAA47708.1, and . CAA47714.1, allele IGHM*03) or SEQ ID NO: 2 (identical to, *e.g.*, GenBank Accession No. sp|P01871.4, allele IGHM*04). The human Cμ1 region ranges from about amino acid 5 to about amino acid 102 of SEQ ID NO: 1 or SEQ ID NO: 2; the human Cμ2 region ranges from about amino acid 114 to about amino acid 205 of SEQ ID NO: 1 or SEQ ID NO: 2, the human Cμ3 region ranges from about amino acid 224 to about amino acid 319 of SEQ ID NO: 1 or SEQ ID NO: 2, the Cμ 4 region ranges from about amino acid 329 to about amino acid

430 of SEQ ID NO: 1 or SEQ ID NO: 2, and the tailpiece ranges from about amino acid 431 to about amino acid 453 of SEQ ID NO: 1 or SEQ ID NO: 2.

**[0130]** Other forms and alleles of the human IgM constant region with minor sequence variations exist, including, without limitation, GenBank Accession Nos. CAB37838.1, and pir||MHHU. The amino acid substitutions, insertions, and/or deletions at positions corresponding to SEQ ID NO: 1 or SEQ ID NO: 2 described and claimed elsewhere in this disclosure can likewise be incorporated into alternate human IgM sequences, as well as into IgM constant region amino acid sequences of other species.

**[0131]** Human IgM constant regions, and also certain non-human primate IgM constant regions, as provided herein typically include five (5) naturally-occurring asparagine (N)-linked glycosylation motifs or sites. See **FIGs. 3A** and **3B**. As used herein "an N-linked glycosylation motif" comprises or consists of the amino acid sequence N-$X_1$-S/T, wherein N is asparagine, $X_1$ is any amino acid except proline (P), and S/T is serine (S) or threonine (T). The glycan is attached to the nitrogen atom of the asparagine residue. *See*, *e.g.*, Drickamer K, Taylor ME (2006), *Introduction to Glycobiology* (2nd ed.). Oxford University Press, USA. N-linked glycosylation motifs occur in the human IgM heavy chain constant regions of SEQ ID NO: 1 or SEQ ID NO: 2 starting at positions 46 ("N1"), 209 ("N2"), 272 ("N3"), 279 ("N4"), and 440 ("N5"). These five motifs are conserved in non-human primate IgM heavy chain constant regions, and four of the five are conserved in the mouse IgM heavy chain constant region. See, *e.g.*, **FIG. 3B**.

**[0132]** Studies on recombinant as well as serum-derived human IgM have shown that the N1, N2, and N3 motifs on the human IgM heavy chain are decorated predominantly, but not invariably with complex-type N-glycans, where the N4 and N5 motifs are decorated predominantly, but not invariably with oligomannose type N-glycans. See, *e.g.*, Moh, E.S.X., *et al.*, *J. Am. Soc. Mass Spectrom. 27*:1143-1155 (2016) and Hennicke, J., *et al.*, *Anal. Biochem. 539*:162-166 (2017).

**[0133]** Each IgM heavy chain constant region can be associated with a binding domain, *e.g.*, an antigen-binding domain, *e.g.*, a scFv or VHH, or a subunit of an antigen-binding domain, *e.g.*, a VH region. In certain embodiments the binding domain can be a non-antibody binding domain, *e.g.*, a receptor ectodomain, a ligand or receptor-binding fragment thereof, a cytokine or receptor-binding fragment thereof, a growth factor or receptor binding fragment thereof, a neurotransmitter or receptor binding fragment thereof, a peptide or protein hormone or receptor binding fragment thereof, an immune checkpoint

modulator ligand or receptor-binding fragment thereof, or a receptor-binding fragment of an extracellular matrix protein. See, *e.g.*, PCT Application Publication No. WO 2020/086745, which is incorporated herein by reference in its entirety.

**[0134]** Five IgM binding units can form a complex with an additional small polypeptide chain (the J-chain), or a functional fragment, variant, or derivative thereof, to form a pentameric IgM antibody or IgM-like antibody. The precursor form of the human J-chain is presented as SEQ ID NO: 5. The signal peptide extends from amino acid 1 to about amino acid 22 of SEQ ID NO: 5, and the mature human J-chain extends from about amino acid 23 to amino acid 159 of SEQ ID NO: 5. The mature human J-chain includes the amino acid sequence SEQ ID NO: 6.

**[0135]** Exemplary variant and modified J-chains are provided elsewhere herein. Without the J-chain, an IgM antibody or IgM-like antibody typically assembles into a hexamer, comprising up to twelve antigen-binding domains. With a J-chain, an IgM antibody or IgM-like antibody typically assembles into a pentamer, comprising up to ten antigen-binding domains, or more, if the J-chain is a modified J-chain comprising one or more heterologous polypeptides comprising additional antigen-binding domain(s). The assembly of five or six IgM binding units into a pentameric or hexameric IgM antibody or IgM-like antibody is thought to involve the Cμ4 and tailpiece domains. *See, e.g.*, Braathen, R., *et al.*, *J. Biol. Chem. 277*:42755-42762 (2002). Accordingly, a pentameric or hexameric IgM antibody provided in this disclosure typically includes at least the Cμ4 and/or tailpiece domains (also referred to herein collectively as Cμ4-tp). A "multimerizing fragment" of an IgM heavy chain constant region thus includes at least the Cμ4-tp domains. An IgM heavy chain constant region can additionally include a Cμ3 domain or a fragment thereof, a Cμ2 domain or a fragment thereof, a Cμ1 domain or a fragment thereof, and/or other IgM heavy chain domains. In certain embodiments, an IgM-derived binding molecule, *e.g.*, an IgM antibody, IgM-like antibody, or other IgM-derived binding molecule as provided herein can include a complete IgM heavy (μ) chain constant domain, *e.g.*, SEQ ID NO: 1 or SEQ ID NO: 2, or a variant, derivative, or analog thereof, *e.g.*, as provided herein.

**[0136]** In certain embodiments, the disclosure provides a monoclonal population of multimeric, *e.g.*, pentameric or hexameric binding molecules, where each binding molecule includes ten or twelve IgM-derived heavy chains, and where the IgM-derived heavy chains comprise glycosylated IgM heavy chain constant regions each associated

with a binding domain that specifically binds to a target. These embodiments are described in detail elsewhere in this disclosure. In certain embodiments, the disclosure provides a monoclonal population of IgM antibodies, IgM-like antibodies, or other IgM-derived binding molecules that include five or six bivalent binding units, where each binding unit includes two IgM or IgM-like heavy chain constant regions or multimerizing fragments or variants thereof, each associated with an antigen-binding domain or subunit thereof. In certain embodiments, the two IgM heavy chain constant regions included in each binding unit are human heavy chain constant regions.

**[0137]** Where the IgM antibody, IgM-like antibody, other IgM-derived binding molecule, or monoclonal population of multimeric binding molecules provided in this disclosure is pentameric, the IgM antibody, IgM-like antibody, other IgM-derived binding molecule, or the molecules contained in a monoclonal population of multimeric binding molecules typically further include a J-chain, or functional fragment or variant thereof. In certain embodiments the J-chain is a modified J-chain or variant thereof that further comprises one or more heterologous moieties attached to the J-chain, as described elsewhere herein. In certain embodiments the J-chain can be mutated to affect, *e.g.*, enhance, the serum half-life of the IgM antibody, IgM-like antibody, other IgM-derived binding molecule, or monoclonal population of multimeric binding molecules provided herein, as discussed elsewhere in this disclosure. In certain embodiments the J-chain can be mutated to affect glycosylation, as discussed elsewhere in this disclosure.

**[0138]** An IgM heavy chain constant region can include one or more of a Cμ1 domain or fragment or variant thereof, a Cμ2 domain or fragment or variant thereof, a Cμ3 domain or fragment or variant thereof, and/or a Cμ4 domain or fragment or variant thereof, provided that the constant region can serve a desired function in the IgM antibody, IgM-like antibody, or other IgM-derived binding molecule, *e.g.*, associate with second IgM constant region to form a binding unit with one, two, or more antigen-binding domain(s), and/or associate with other binding units (and in the case of a pentamer, a J-chain) to form a hexamer or a pentamer. In certain embodiments the two IgM heavy chain constant regions or fragments or variants thereof within an individual binding unit each comprise a Cμ4 domain or fragment or variant thereof, a tailpiece (tp) or fragment or variant thereof, or a combination of a Cμ4 domain and a TP or fragment or variant thereof. In certain embodiments the two IgM heavy chain constant regions or fragments or variants thereof within an individual binding unit each further comprise a Cμ3 domain or fragment or

variant thereof, a Cμ2 domain or fragment or variant thereof, a Cμ1 domain or fragment or variant thereof, or any combination thereof.

Modified J-chains

**[0139]** In certain embodiments, the J-chain of a pentameric IgM-derived binding molecule, *e.g.*, an IgM antibody or IgM-like antibody as provided herein can be modified, *e.g.*, by introduction of a heterologous moiety, or two or more heterologous moieties, *e.g.*, polypeptides, without interfering with the ability of IgM antibody, IgM-like antibody, other IgM-derived binding molecule, or monoclonal population of multimeric binding molecules to assemble and bind to its binding target(s). *See* U.S. Patent Nos. 9,951,134, 10,400,038, and 10,618,978, and U.S. Patent Application Publication No. US-2019-0185570, each of which is incorporated herein by reference in its entirety. Accordingly, an IgM antibody, IgM-like antibody, other IgM-derived binding molecule, or monoclonal population of multimeric binding molecules provided by this disclosure, including multispecific IgM or IgM-like antibodies as described elsewhere herein, can comprise a modified J-chain or functional fragment or variant thereof comprising a heterologous moiety, *e.g.*, a heterologous polypeptide, introduced, *e.g.*, fused or chemically conjugated, into the J-chain or fragment or variant thereof. In certain embodiments the heterologous moiety can be a peptide or polypeptide sequence fused in frame to the J-chain or chemically conjugated to the J-chain or fragment or variant thereof. In certain embodiments, the heterologous polypeptide is fused to the J-chain or functional fragment thereof via a peptide linker, *e.g.*, a peptide linker, typically consisting of least 5 amino acids, but no more than 25 amino acids. In certain embodiments, the peptide linker consists of GGGGS (SEQ ID NO: 41), GGGGSGGGGS (SEQ ID NO: 42), GGGGSGGGGSGGGGS (SEQ ID NO: 43), GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 44), or GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 45). In certain embodiments the heterologous moiety can be a chemical moiety conjugated to the J-chain. Heterologous moieties to be attached to a J-chain can include, without limitation, a binding moiety, *e.g.*, an antibody or antigen-binding fragment thereof, *e.g.*, a single chain Fv (scFv) molecule, a cytokine, *e.g.*, IL-2 or IL-15 (see, *e.g.*, PCT Application Publication No. WO 2020/086745, which is incorporated herein by reference in its entirety), a stabilizing peptide that can increase the half-life of the IgM antibody, IgM-like antibody, other IgM-derived binding molecule, or monoclonal population of multimeric binding molecules,

*e.g.*, human serum albumin (HSA) or an HSA binding molecule, or a heterologous chemical moiety such as a polymer or a cytotoxin.

**[0140]** In some embodiments, a modified J-chain can comprise an antigen-binding domain that can include without limitation a polypeptide capable of specifically binding to a target antigen. In certain embodiments, an antigen-binding domain associated with a modified J-chain can be an antibody or an antigen-binding fragment thereof, as described elsewhere herein. In certain embodiments the antigen-binding domain can be a scFv antigen-binding domain or a single-chain antigen-binding domain derived, *e.g.*, from a camelid or condricthoid antibody. The antigen-binding domain can be introduced into the J-chain at any location that allows the binding of the antigen-binding domain to its binding target without interfering with J-chain function or the function of an associated IgM or IgA antibody. Insertion locations include but are not limited to at or near the C-terminus, at or near the N-terminus or at an internal location that, based on the three-dimensional structure of the J-chain, is accessible. In certain embodiments, the antigen-binding domain can be introduced into the mature human J-chain of SEQ ID NO: 6 between cysteine residues 92 and 101 of SEQ ID NO: 6. In a further embodiment, the antigen-binding domain can be introduced into the human J-chain of SEQ ID NO: 6 at or near a glycosylation site. In a further embodiment, the antigen-binding domain can be introduced into the human J-chain of SEQ ID NO: 6 within about 10 amino acid residues from the C-terminus, or within about 10 amino acids from the N-terminus.

**[0141]** In certain embodiments, the J-chain of the IgM antibody, IgM-like antibody, or other IgM-derived binding molecule as provided herein comprises an amino acid substitution at the amino acid position corresponding to amino acid Y102 of the mature wild-type human J-chain (SEQ ID NO: 6). By "an amino acid corresponding to amino acid Y102 of the mature wild-type human J-chain" is meant the amino acid in the sequence of the J-chain of any species which is homologous to Y102 in the human J-chain. See US Patent Application Publication No. US 2020-0239572, which is incorporated herein by reference in its entirety. The position corresponding to Y102 in SEQ ID NO: 6 is conserved in the J-chain amino acid sequences of at least 43 other species. *See* FIG. 4 of U.S. Patent No. 9,951,134, which is incorporated by reference herein. Certain mutations at the position corresponding to Y102 of SEQ ID NO: 6 can inhibit the binding of certain immunoglobulin receptors, *e.g.*, the human or murine Fcαμ receptor, the murine Fcμ receptor, and/or the human or murine polymeric Ig receptor (pIg receptor) to an IgM

pentamer comprising the mutant J-chain. An IgM antibody, IgM-like antibody, other IgM-derived binding molecule, or monoclonal population of multimeric binding molecules comprising a mutation at the amino acid corresponding to Y102 of SEQ ID NO: 6 has an improved serum half-life when administered to an animal than a corresponding antibody, antibody-like molecule, binding molecule, or monoclonal population of binding molecules that is identical except for the substitution, and which is administered to the same species in the same manner. In certain embodiments, the amino acid corresponding to Y102 of SEQ ID NO: 6 can be substituted with any amino acid. In certain embodiments, the amino acid corresponding to Y102 of SEQ ID NO: 6 can be substituted with alanine (A), serine (S) or arginine (R). In a particular embodiment, the amino acid corresponding to Y102 of SEQ ID NO: 6 can be substituted with alanine. In a particular embodiment the J-chain or functional fragment or variant thereof is a variant human J-chain referred to herein as "J*," and comprises the amino acid sequence SEQ ID NO: 7.

Highly sialylated populations of IgM-derived binding molecules

**[0142]** This disclosure provides a monoclonal population of multimeric binding molecules, where each binding molecule comprises ten or twelve IgM-derived heavy chains, each comprising a glycosylated IgM heavy chain constant region, or multimerizing fragment thereof associated with a binding domain that specifically binds to a target. In certain embodiments, 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 comprises the amino acid sequence N-$X_1$-S/T, where N is asparagine, $X_1$ is any amino acid except proline, and S/T is serine or threonine. In certain embodiments, at least one, at least two, at least three, at least four, or at least five of the N-linked glycosylation motifs on each IgM heavy chain constant region are occupied by a complex glycan as defined elsewhere herein. While a human or non-human primate IgM heavy chain constant region typically includes five N-linked glycosylation motifs N1 to N5, as noted earlier N4 and N5 are typically, but not invariably, occupied by oligomannose-type oligosaccharides as opposed to complex oligosaccharides. Thus, in certain embodiments, at least three of the N-linked glycosylation motifs (*e.g.*, N1, N2, and N3) on each IgM heavy chain constant region are occupied by a complex glycan.

**[0143]** In certain embodiments, the monoclonal population of binding molecules provided by this disclosure comprises a level of sialylation that is greater than that observed or

measured for IgM antibodies in normal circulation, *i.e.*, the provided monoclonal population of binding molecules includes a non-naturally-occurring level of sialylation. As measured by the inventors (see, *e.g.*, Example 4), the average level of sialylation for human IgM antibodies isolated from normal circulation is about 30-32 moles sialic acid per mole of IgM. Accordingly, this disclosure provides a monoclonal population of multimeric binding molecules as noted above, that includes at least thirty-three (33), at least thirty-four (34), or at least of at least thirty-five (35) moles sialic acid per mole of the binding molecules. Sialic acid residues are typically the terminal monosaccharides on complex glycans, and a single oligosaccharide glycan can include, *e.g.*, one, two, three, or four sialic acid monosaccharides depending on the number of antennae on the oligosaccharide. In certain embodiments the provided monoclonal population of binding molecules can include higher levels of sialylation, *e.g.*, the monoclonal population of binding molecules can include at least 40, at least 45, 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 146 moles sialic acid per mole of binding molecule. In some embodiments, the monoclonal population of binding molecules includes 33-146 moles sialic acid per mole of binding molecule, such as 33-130, 33-120, 33-110, 33-100, 33-90, 33-80, 33-70, 33-60, 33-50, 35-130, 35-120, 35-110, 35-100, 35-90, 35-80, 35-70, 35-60, 35-50, 45-130, 45-120, 45-110, 45-100, 45-90, 45-80, 45-70, 45-60, 45-50, 50-130, 50-120, 50-110, 50-100, 50-90, 50-80, 50-70, or 50-60 moles sialic acid per mole of binding molecule. In some embodiments, the monoclonal population of binding molecules comprises about 35 to about 40, about 35 to about 45, about 35 to about 50, about 35 to about 55, about 35 to about 60, about 35 to about 65, about 35 to about 70, about 40 to about 45, about 40 to about 50, about 40 to about 55, about 40 to about 60, about 40 to about 65, about 40 to about 70, about 45 to about 50, about 45 to about 55, about 45 to about 60, about 45 to about 65, about 45 to about 70, about 50 to about 55, about 50 to about 60, about 50 to about 65, about 50 to about 70, about 55 to about 60, about 55 to about 65, about 55 to about 70, about 60 to about 65, about 60 to about 70, or about 65 to about 70 moles sialic acid per mole of binding molecule. In some embodiments, the monoclonal population of binding molecules comprises about 40 to about 55 moles sialic acid per mole of binding molecule. As demonstrated in the examples herein, monoclonal population of binding molecules with sialic acid levels above 35 moles of sialic acid per mole of binding molecule have improved pharmacokinetic properties of the binding molecules compared

to the same binding molecules with lower levels of sialic acid. For certain instances, it may be desirable to make and use a monoclonal population of binding molecules, wherein the sialic level is not at the maximum possible level, such about 40 to about 55 moles sialic acid per mole of binding molecule. Such molecules may have other desirable properties, such as a different solubility, ease of manufacturability, and/or immunogenicity.

**[0144]** As provided by this disclosure, each IgM-derived heavy chain in the provided population of binding molecules includes a glycosylated IgM or IgM-derived heavy chain constant region or multimerizing fragment or derivative thereof, which can be a full-length IgM heavy chain constant region, a multimerizing fragment of an IgM heavy chain constant region, or a hybrid constant region that includes at least the minimal portion of an IgM heavy chain constant region required for multimerization, associated with a binding domain, *e.g.*, an antibody antigen-binding domain, that specifically binds to a target of interest. In certain embodiments, the IgM heavy chain constant regions are derived from human IgM heavy chain constant regions that include up to five N-linked glycosylation motifs N-$X_1$-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). The binding domain that binds to a target can be, *e.g.*, an antigen-binding domain or a subunit of an antigen-binding domain, *e.g.*, the heavy chain variable region (VH) of an antibody. This disclosure relates to binding molecules that bind to any target of interest.

**[0145]** The monoclonal population of binding molecules provided by this disclosure can be produced in a number of different ways, including, but not limited to, modifications to the cell line expressing the population of binding molecules, by *in vitro* glycoengineering of the monoclonal population of binding molecules during downstream processing, or any combination of these, or other methods.

**[0146]** In certain embodiments, the provided highly sialylated monoclonal population of multimeric binding molecules is produced via cell line modification. Cell line modifications to increase sialylation of a monoclonal population of binding molecules as provided by this disclosure include, without limitation, transfecting the cell line that produces the monoclonal population of binding molecules with one or more genes encoding glycosyltransferases, *e.g.*, galactosyltransferases (to provide an acceptor residue for a sialic acid residue via an alpha-2,6 and/or alpha-2,3 linkage, see, *e.g.*, **FIGs. 1C** and

1D) and/or or more sialyltransferases to produce cell lines that overexpress these enzymes (glycosyltransferase "knock-ins"), thereby improving and/or increasing the capacity of the ability of the cell line to facilitate the transfer of sialic acid monosaccharides from a CMP-NANA substrate or a derivative thereof to a compatible acceptor oligosaccharide. Other cell line modifications include the deletion or "knock-out" of sialidase enzymes normally produced by the cell line. Methods for "knocking in" various glucosyltransferases are described in the examples and are otherwise well known by persons of ordinary skill in the art. Likewise, methods for "knocking out" genes encoding, *e.g.*, sialidases in a cell line are readily available to the skilled person.

**[0147]** An exemplary sialyltransferase is human beta-galactoside alpha-2,6-sialyltransferase 1, also referred to as ST6GAL1 (SEQ ID NO: 3). Other sialyltransferases that can be "knocked in" include human beta-galactoside alpha-2,6-sialyltransferase-II (ST6GALII), and any of the four beta-galactoside α2–3-sialyltransferases (ST3GAL-I-IV). An exemplary galactosyltransferase is human beta-1,4-galactosyltransferase 4 (B4GALT4) (SEQ ID NO: 4).

**[0148]** In certain embodiments, the provided highly sialylated monoclonal population of multimeric binding molecules is produced via glycoengineering, to produce, *e.g.*, a monoclonal population of glycoengineered IgM antibodies, IgM-like antibodies, or IgM-derived binding molecules (GEMs), *e.g.*, via the addition of sialic acid residues to the monoclonal population of binding molecules during downstream processing. In certain embodiments, the *in vitro* glycoengineering comprises contacting the monoclonal population of binding molecules with a soluble sialyltransferase (or a soluble sialyltransferase attached to a solid support) and a sialic acid substrate (*e.g.*, a substrate comprising cytidine monophosphate (CMP)-N-acetyl-neuraminic acid (CMP-NANA)), under conditions where sialic acid is transferred from CMP-NANA to a galactose residue on complex glycans on the population of binding molecules. The contacting can occur during one or more steps of protein purification, after which the soluble sialyltransferase can be removed by a subsequent purification step, or by separating the population of binding molecules from the solid support to which the enzyme is attached.

**[0149]** In certain embodiments the sialyltransferase variant to be used in production of GEMs can be a soluble variant of human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) (SEQ ID NO: 3). For example, the sialyltransferase can be a variant of ST6GAL1 that excludes the transmembrane region of SEQ ID NO: 3 (*e.g.*, excluding

amino acids 10 to 26 of SEQ ID NO: 3), or both the cytoplasmic and transmembrane regions of SEQ ID NO: 3 (*e.g.*, excluding amino acids 1 to 9 of SEQ ID NO: 3 and amino acids 10 to 26 of SEQ ID NO: 3), but maintains the catalytic activity of the protein. In certain embodiments, the soluble variant of ST6GAL1 comprises amino acids x to 406 of SEQ ID NO: 3, wherein x is an integer from 27 to 120. For example the soluble variant of ST6GAL1 can include 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 certain embodiments the sialic acid substrate comprises cytidine monophosphate (CMP)-N-acetyl-neuraminic acid (=CMP-β-D-Neu5Ac; =CMP-Neu5Ac; =CMP-NANA; =CMP-sialic acid; =CMP-SA, **FIG. 2B**). Functional derivatives include but are not limited to azido-CMP-sialic acid to be used for glycan labelling by "click" chemistry.

**[0150]** The inventors have observed that despite the large numbers of glycans (51 for pentamers, 60 for hexamers), efficient and high-level sialylation of IgM antibodies can be carried out with low concentrations of the soluble variant of ST6GAL1, relative to the higher amounts required for glycoengineering of IgG antibodies. For example, efficient sialylation of IgM antibodies has been carried out with a mass ratio of IgM antibody to soluble sialyltransferase of about 5000:1 or 2000:1, and an IgM antibody to sialic acid substrate to soluble sialyltransferase ratio of about 5000:2500:1 or 2000:500:1 (an excess amount of the sialic acid substrate is provided). This calculates to a molar ratio of IgM antibody to sialyltransferase of about 200:1 or 80:1 or a molar ratio of IgM antibody to sialic acid substrate to sialyltransferase of about 200:2500:1 or 80:500:1. In certain embodiments the molar ratio of IgM antibody to sialyltransferase is at least about 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 90:1, 95:1, 100:1, 105:1, 110:1, 115:1, 120:1, 125:1, 130:1, 135:1, 140:1, 145:1, 150:1, 175:1, or 200:1. In some embodiments, the mass ratio of binding molecule: sialyltransferase can be about 80:1 to about 5000:1. In some embodiments, the mass ratio of binding molecule: sialyltransferase can be about 80:1 to about 100:1, about 80:1 to about 250:1, about 80:1 to about 500:1, about 80:1 to about 750:1, about 80:1 to about 1000:1, about 80:1 to about 1250:1, about 80:1 to about 1500:1, about 80:1 to about 1750:1, about 80:1 to about 2000:1, about 80:1 to about 2500:1, about 80:1 to about 3000:1, about 80:1 to about 3500:1, about 80:1 to about 4000:1, about 80:1

to about 4500:1, about 250:1 to about 500:1, about 250:1 to about 750:1, about 250:1 to about 1000:1, about 250:1 to about 1250:1, about 250:1 to about 1500:1, about 250:1 to about 1750:1, about 250:1 to about 2000:1, about 250:1 to about 2500:1, about 250:1 to about 3000:1, about 250:1 to about 3500:1, about 250:1 to about 4000:1, about 250:1 to about 4500:1, about 250:1 to about 5000:1, about 500:1 to about 750:1, about 500:1 to about 1000:1, about 500:1 to about 1250:1, about 500:1 to about 1500:1, about 500:1 to about 1750:1, about 500:1 to about 2000:1, about 500:1 to about 2500:1, about 500:1 to about 3000:1, about 500:1 to about 3500:1, about 500:1 to about 4000:1, about 500:1 to about 4500:1, about 500:1 to about 5000:1, about 1000:1 to about 1250:1, about 1000:1 to about 1500:1, about 1000:1 to about 1750:1, about 1000:1 to about 2000:1, about 1000:1 to about 2500:1, about 1000:1 to about 3000:1, about 1000:1 to about 3500:1, about 1000:1 to about 4000:1, about 1000:1 to about 4500:1, about 1000:1 to about 5000:1, about 1500:1 to about 1750:1, about 1500:1 to about 2000:1, about 1500:1 to about 2500:1, about 1500:1 to about 3000:1, about 1500:1 to about 3500:1, about 1500:1 to about 4000:1, about 1500:1 to about 4500:1, about 1500:1 to about 5000:1, about 2000:1 to about 2500:1, about 2000:1 to about 3000:1, about 2000:1 to about 3500:1, about 2000:1 to about 4000:1, about 2000:1 to about 4500:1, about 2000:1 to about 5000:1, about 2500:1 to about 3000:1, about 2500:1 to about 3500:1, about 2500:1 to about 4000:1, about 2500:1 to about 4500:1, about 2500:1 to about 5000:1, about 3000:1 to about 3500:1, about 3000:1 to about 4000:1, about 3000:1 to about 4500:1, about 3000:1 to about 5000:1, about 3500:1 to about 4000:1, about 3500:1 to about 4500:1, about 3500:1 to about 5000:1, about 4000:1 to about 4500:1, or about 4000:1 to about 5000:1. This is in contrast to the much larger amounts of enzyme required for *in vitro* sialylation of IgG antibodies, with a recommended molar ratio of IgG antibody to sialyltransferase of 3:1. See, *e.g.*, Malik, S., and Thomann, M., (2016) *In Vitro* Glycoengineering – Suitability for BioPharma manufacturing, Application Note, available custombiotech.roche.com.

**[0151]** The inventors have also observed that despite the large numbers of glycans (51 for pentamers, 60 for hexamers), efficient and high-level sialylation of IgM antibodies can be carried out with low concentrations of the sialic acid substrate, relative to the higher amounts required for glycoengineering of IgG antibodies. In some embodiments, the mass ratio of sialic acid substrate: sialyltransferase can be about 1:4 to about 3000:1, such as about 1:4 to about 1:1, about 1:4 to about 5:1, about 1:4 to about 50:1, about 1:4 to about 100:1, about 1:4 to about 500:1, about 1:4 to about 1000:1, about 1:4 to about 1500:1,

about 1:4 to about 2000:1, about 1:4 to about 2500:1, about 1:1 to about 5:1, about 1:1 to about 10:1, about 1:1 to about 50:1, about 1:1 to about 100:1, about 1:1 to about 500:1, about 1:1 to about 1000:1, about 1:1 to about 1500:1, about 1:1 to about 2000:1, about 1:1 to about 2500:1, about 1:1 to about 3000:1, about 2:1 to about 5:1, about 2:1 to about 10:1, about 2:1 to about 50:1, about 2:1 to about 100:1, about 2:1 to about 500:1, about 2:1 to about 1000:1, about 2:1 to about 1500:1, about 2:1 to about 2000:1, about 2:1 to about 2500:1, about 2:1 to about 3000:1, about 5:1 to about 10:1, about 5:1 to about 50:1, about 5:1 to about 100:1, about 5:1 to about 500:1, about 5:1 to about 1000:1, about 5:1 to about 1500:1, about 5:1 to about 2000:1, about 5:1 to about 2500:1, about 5:1 to about 3000:1, about 10:1 to about 50:1, about 10:1 to about 100:1, about 10:1 to about 500:1, about 10:1 to about 1000:1, about 10:1 to about 1500:1, about 10:1 to about 2000:1, about 10:1 to about 2500:1, about 10:1 to about 3000:1, about 50:1 to about 100:1, about 50:1 to about 500:1, about 50:1 to about 1000:1, about 50:1 to about 1500:1, about 50:1 to about 2000:1, about 50:1 to about 2500:1, about 50:1 to about 3000:1, about 100:1 to about 500:1, about 100:1 to about 1000:1, about 100:1 to about 1500:1, about 100:1 to about 2000:1, about 100:1 to about 2500:1, about 100:1 to about 3000:1, about 500:1 to about 1000:1, about 500:1 to about 1500:1, about 500:1 to about 2000:1, about 500:1 to about 2500:1, about 500:1 to about 3000:1, about 1000:1 to about 1500:1, about 1000:1 to about 2000:1, about 1000:1 to about 2500:1, about 1000:1 to about 3000:1, about 1500:1 to about 2000:1, about 1500:1 to about 2500:1, about 1500:1 to about 3000:1, about 2000:1 to about 2500:1, about 2000:1 to about 3000:1, or about 2500:1 to about 3000:1. In some embodiments, the mass ratio of binding molecule: sialyltransferase can be about 80:1, about 100:1, about 250:1, about 500:1, about 750:1, about 1000:1, about 1250:1, about 1500:1, about 1750:1, about 2000:1, about 2500:1, about 3000:1, about 3500:1, about 4000:1, about 4500:1, or about 5000:1; and/or the mass ratio of sialic acid substrate: sialyltransferase can be about 5:1, about 10:1, about 50:1, about 100:1, about 500:1, about 1000:1, about 1500:1, about 2000:1, about 2500:1, or about 3000:1.

**[0152]** In some embodiments, the mass ratio of antibody: sialic acid substrate can be about 1:1 to about 40:1, such as about 1:1 to about 2:1, about 1:1 to about 4:1, about 1:1 to about 6:1, about 1:1 to about 8:1, about 1:1 to about 10:1, about 1:1 to about 15:1, about 1:1 to about 20:1, about 2:1 to about 4:1, about 2:1 to about 6:1, about 2:1 to about 8:1, about 2:1 to about 10:1, about 2:1 to about 15:1, about 2:1 to about 20:1, about 2:1 to about 40:1, about 4:1 to about 6:1, about 4:1 to about 8:1, about 4:1 to about 10:1, about 4:1 to about

15:1, about 4:1 to about 20:1, about 4:1 to about 40:1, about 6:1 to about 8:1, about 6:1 to about 10:1, about 6:1 to about 15:1, about 6:1 to about 20:1, about 6:1 to about 40:1, about 8:1 to about 10:1, about 8:1 to about 15:1, about 8:1 to about 20:1, about 8:1 to about 40:1, about 10:1 to about 15:1, about 10:1 to about 20:1, about 10:1 to about 40:1, about 15:1 to about 20:1, about 15:1 to about 40:1, or about 20:1 to about 40:1.

**[0153]** The inventors have further observed that efficient and high-level sialylation of IgM antibodies can be carried out at a greater range of temperatures and for longer periods of time than what was used for glycoengineering of IgG antibodies. In some embodiments, the *in vitro* glycoengineering comprises contacting the monoclonal population of binding molecules with a soluble sialyltransferase and a sialic acid substrate for at least 30 minutes, such as at least 45 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 10 hours, at least 12 hours, at least 18 hours, at least 24 hours, at least 36 hours, or at least 48 hours. In some embodiments, the contacting occurs for about 30 minutes to about 48 hours, such as about 30 minutes to about 4 hours, about 30 minutes to about 5 hours, about 30 minutes to about 6 hours, about 30 minutes to about 7 hours, about 30 minutes to about 10 hours, about 30 minutes to about 12 hours, about 30 minutes to about 18 hours, about 30 minutes to about 24 hours, about 30 minutes to about 36 hours, about 2 hours to about 48 hours, about 3 hours to about 6 hours, about 3 hours to about 10 hours, about 3 hours to about 12 hours, about 3 hours to about 18 hours, about 3 hours to about 24 hours, about 3 hours to about 36 hours, about 3 hours to about 48 hours, about 4 hours to about 10 hours, about 4 hours to about 12 hours, about 4 hours to about 18 hours, about 4 hours to about 24 hours, about 4 hours to about 36 hours, about 4 hours to about 48 hours, about 5 hours to about 10 hours, about 5 hours to about 12 hours, about 5 hours to about 18 hours, about 5 hours to about 24 hours, about 5 hours to about 36 hours, about 5 hours to about 48 hours, about 7 hours to about 10 hours, about 7 hours to about 12 hours, about 7 hours to about 18 hours, about 7 hours to about 24 hours, about 7 hours to about 36 hours, about 7 hours to about 48 hours, about 10 hours to about 18 hours, about 10 hours to about 24 hours, about 10 hours to about 36 hours, about 10 hours to about 48 hours, about 12 to about 18 hours, about 12 hours to about 24 hours, about 12 hours to about 36 hours, about 12 hours to about 48 hours, about 18 hours to about 24 hours, about 18 hours to about 36 hours, about 18 hours to about 48 hours, about 24 hours to about 36 hours, about 24 hours to about 48 hours, or about 36 hours to about 48 hours,

**[0154]** In some embodiments, the *in vitro* glycoengineering comprises contacting the monoclonal population of binding molecules with a soluble sialyltransferase and a sialic acid substrate at a temperature at about 2 °C to about 40 °C, such as at about 2 °C to about 37 °C, 2 °C to about 30 °C, 2 °C to about 25 °C, 2 °C to about 22 °C, 2 °C to about 20 °C, 2 °C to about 10 °C, about 4 °C to about 40 °C, about 4 °C to about 37 °C, 4 °C to about 30 °C, 4 °C to about 25 °C, 4 °C to about 22 °C, 4 °C to about 20 °C, 4 °C to about 10 °C, about 10 °C to about 40 °C, about 10 °C to about 37 °C, 10 °C to about 30 °C, 10 °C to about 25 °C, 10 °C to about 22 °C, 10 °C to about 20 °C, about 20 °C to about 40 °C, about 20 °C to about 37 °C, 20 °C to about 30 °C, 20 °C to about 25 °C, 20 °C to about 22 °C, about 22 °C to about 40 °C, about 22 °C to about 37 °C, 22 °C to about 30 °C, 22 °C to about 25 °C, about 25 °C to about 40 °C, about 25 °C to about 37 °C, 25 °C to about 30 °C, about 30 °C to about 40 °C, or about 30 °C to about 37 °C. In some embodiments, the *in vitro* glycoengineering comprises contacting the monoclonal population of binding molecules with a soluble sialyltransferase and a sialic acid substrate at a temperature at about 15 °C to about 25 °C.

**[0155]** In certain embodiments, *in vitro* sialylation can be enhanced by ensuring a sufficient number of galactose acceptor residues are present on the complex glycans of the provided monoclonal population of IgM, IgM-like, or IgM-derived binding molecules. ST6GAL1 transfers a sialic acid monosaccharide from CMP-NANA to a galactose acceptor residue on the molecule's glycans via an α-2,6 linkage. To insure a sufficient number of acceptor galactose residues on the glycans present in the monoclonal population of binding molecules, production of GEMs can further include contacting the monoclonal population of binding molecules with a galactosyltransferase, *e.g.*, a soluble variant of beta-1,4-galactosyltransferase 4 (B4GALT4) (SEQ ID NO: 4), and a galactose substrate, *e.g.*, uridine-diphosphate-α-D-galactose (UDP-Gal), either prior to or simultaneously with the contacting with the sialyltransferase and sialic acid substrate. For example, the sialyltransferase can be a variant of B4GALT4 that excludes the transmembrane region of SEQ ID NO: 4 (*e.g.*, excluding amino acids 13 to 38 of SEQ ID NO: 4), or both the cytoplasmic and transmembrane regions of SEQ ID NO: 4 (*e.g.*, excluding amino acids 1 to 12 of SEQ ID NO: 4 and amino acids 13 to 38 of SEQ ID NO: 4), but maintains the catalytic activity of the protein. In certain embodiments the soluble variant of B4GALT4 comprises amino acids x to 344 of SEQ ID NO: 4, wherein x is an integer from 39 to 120. For example, the soluble variant of B4GALT4 comprises 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 certain embodiments the galactose substrate comprises UDP-Gal.

**[0156]** IgM heavy chain constant regions in the provided monoclonal population of binding molecules are each associated with a binding domain or subunit thereof, *e.g.*, an antibody antigen-binding domain, *e.g.*, a scFv, a VHH or the VH subunit of an antibody antigen-binding domain, where the binding domain specifically binds to a target of interest. In certain embodiments, the target is a target epitope, a target antigen, a target cell, a target organ, or a target virus. Targets can include, without limitation, tumor antigens, other oncologic targets, immuno-oncologic targets such as immune checkpoint inhibitors, infectious disease targets, such as viral antigens expressed on the surface of infected cells, target antigens involved in blood-brain-barrier transport, target antigens involved in neurodegenerative diseases and neuroinflammatory diseases, and any combination thereof. Exemplary targets and binding domains that bind to such targets are provided elsewhere herein, and can be found in, *e.g.*, U.S. Patent Application Publication Nos. US 2019-0330360, US 2019-0338040, US 2019-0338041, US 2019-0330374, US 2019-0185570, US 2019-0338031, or US 2020-0239572, in PCT Publication Nos. WO 2018/017888, WO 2018/017889, WO 2018/017761, WO 2018/017763, or WO 2018/187702, and WO 2019/165340, or in U.S. Patent Nos. 9,951,134, 9,938,347, 8,377,435, 9,458,241, 9,409,976, 10,400,038, 10,351,631, 10,570,191, 10,604,559, 10,618,978, 10,689,449, or 10,787,520. Each of these applications and/or patents are incorporated herein by reference in their entireties.

**[0157]** In certain embodiments, the provided population of multimeric binding molecules is multispecific, *e.g.*, bispecific, trispecific, or tetraspecific, where two or more binding domains associated with the IgM heavy chain constant regions of each binding molecule specifically bind to different targets. In certain embodiments, the binding domains of the provided population of multimeric binding molecules all specifically bind to the same target. In certain embodiments, the binding domains of the provided population of multimeric binding molecules are identical. In such cases the population of multimeric binding molecules can still be bispecific, if, for example, a binding domain with a different specificity is part of a modified J-chain as described elsewhere herein. In certain embodiments, the binding domains are antibody-derived antigen-binding domains, *e.g.*, a

scFv associated with the IgM heavy chain constant regions or a VH subunit of an antibody binding domain associated with the IgM heavy chain constant regions.

**[0158]** In certain embodiments, 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. In certain embodiments each antigen-binding domain of each binding molecule binds to the same target. In certain embodiments, each antigen-binding domain of each binding molecule is identical.

**[0159]** In certain embodiments, the target is a tumor-specific antigen, *i.e.*, a target antigen that is largely expressed only on tumor or cancer cells, or that may be expressed only at undetectable levels in normal healthy cells of an adult. In certain embodiments, the target is a tumor-associated antigen, *i.e.*, a target antigen that is expressed on both healthy and cancerous cells but is expressed at a much higher density on cancerous cells than on normal healthy cells. Exemplary tumor-specific or tumor-associated antigens include, without limitation, B-cell maturation antigen (BCMA), CD19, CD20, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2, also called ErbB2), HER3 (ErbB3), receptor tyrosine-protein kinase ErbB4, cytotoxic T-lymphocyte antigen 4 (CTLA4), programmed cell death protein 1 (PD-1), Programmed death-ligand 1 (PD-L1), vascular endothelial growth factor (VEGF), VEGF receptor-1 (VEGFR1), VEGFR2, CD52, CD30, prostate-specific membrane antigen (PSMA), CD38, ganglioside GD2, self-ligand receptor of the signaling lymphocytic activation molecule family member 7 (SLAMF7), platelet-derived growth factor receptor A (PDGFRA), CD22, FLT3 (CD135), CD123, MUC-16, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM-1), mesothelin, tumor-associated calcium signal transducer 2 (Trop-2), glypican-3 (GPC-3), human blood group H type 1 trisaccharide (Globo-H), sialyl Tn antigen (STn antigen), and CD33. The skilled person will understand that these target antigens appear in the literature by a number of different names, but that these therapeutic targets can be easily identified using databases available online, *e.g.*, EXPASY_dot_org.

**[0160]** Other tumor associated or tumor-specific antigens include, without limitation: DLL4, Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, c-Met, IGF-1R, Patched,

Hedgehog family polypeptides, WNT family polypeptides, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, FZD10, LRP5, LRP6, IL-6, TNFalpha, IL-23, IL-17, CD80, CD86, CD3, CEA, Muc16, PSCA, CD44, c-Kit, DDR1, DDR2, RSPO1, RSPO2, RSPO3, RSPO4, BMP family polypeptides, BMPR1a, BMPR1b, or a TNF receptor superfamily protein such as TNFR1 (DR1), TNFR2, TNFR1/2, CD40 (p50), Fas (CD95, Apo1, DR2), CD30, 4-1BB (CD137, ILA), TRAILR1 (DR4, Apo2), DR5 (TRAILR2), TRAILR3 (DcR1), TRAILR4 (DcR2), OPG (OCIF), TWEAKR (FN14), LIGHTR (HVEM), DcR3, DR3, EDAR, and XEDAR.

**[0161]** In certain embodiments, the monoclonal population of multimeric binding molecules comprises a population of pentameric or hexameric IgM antibodies, IgM-like antibodies, or other IgM-derived binding molecules that each include five or six bivalent IgM binding units, respectively. According to certain embodiments, each binding unit includes two IgM heavy chains as described herein, each having a VH situated amino terminal to the variant IgM constant region, and two immunoglobulin light chains each having a light chain variable domain (VL) situated amino terminal to an immunoglobulin light chain constant region, *e.g.*, a kappa or lambda constant region. The provided VH and VL combine to form an antigen-binding domain that specifically binds to the target of interest. In certain embodiments, the five or six IgM binding units are identical.

**[0162]** In those embodiments where the population of multimeric IgM antibodies, IgM-like antibodies, or IgM-derived binding molecules is pentameric, each antibody or binding molecule can further include a J-chain, or functional fragment thereof, or a functional variant thereof, as described elsewhere herein. For example, the J-chain can be a mature human J-chain that includes the amino acid sequence SEQ ID NO: 6 or a functional fragment thereof, or a functional variant thereof. As persons of ordinary skill in the art will recognize, "a functional fragment" or a "functional variant" in this context includes those fragments and variants that can associate with IgM binding units, *e.g.*, IgM heavy chain constant regions, to form a pentameric IgM antibody, IgM-like antibody, or IgM-derived binding molecule.

**[0163]** In certain embodiments, the J-chain of a pentameric IgM-derived binding molecule, *e.g.*, an IgM antibody, IgM-like antibody, or other IgM-derived binding molecule as provided herein is a functional variant J-chain that includes 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.

For example, certain amino acid substitutions, deletions, or insertions can result in the IgM-derived binding molecule exhibiting 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 certain embodiments the variant J-chain can include one, two, three, or four single amino acid substitutions, deletions, or insertions relative to the reference J-chain.

**[0164]** As described in detail elsewhere herein, in certain embodiments the variant J-chain or functional fragment thereof of a pentameric IgM-derived binding molecule, *e.g.*, an IgM antibody, IgM-like antibody, or other IgM-derived binding molecule as provided herein comprises an amino acid substitution at the amino acid position corresponding to amino acid Y102 of the wild-type mature human J-chain (SEQ ID NO: 6). Y102 can be substituted with any amino acid, for example alanine. In certain embodiments the variant human J-chain can include the amino acid sequence SEQ ID NO: 7. A J-chain having the amino acid sequence of SEQ ID NO: 7 can in some instances be referred to as "J*."

**[0165]** The J-chain or fragment of a pentameric IgM-derived binding molecule, *e.g.*, an IgM antibody, IgM-like antibody, or other IgM-derived binding molecule as provided herein, having either a variant or wild type amino acid sequence, can be a "modified J-chain" that further include a heterologous moiety, wherein the heterologous moiety is fused or conjugated to the J-chain or fragment or variant thereof. Exemplary, but non-limiting heterologous moieties are provided, *e.g.*, in U.S. Patent Nos. 9,951,134 and 10,618,978, and in U.S. Patent Application Publication No. 2019/0185570, which are incorporated herein by reference. In certain embodiments, the heterologous moiety is a polypeptide fused to or within the J-chain or fragment or variant thereof. The heterologous polypeptide can in some instances be fused to or within the J-chain or fragment or variant thereof via a peptide linker. Any suitable linker can be used, for example the peptide linker can include at least 5 amino acids, at least ten amino acids, and least 20 amino acids, at least 30 amino acids or more, and so on. In certain embodiments the peptide linker includes no more than 25 amino acids. In certain embodiments the peptide linker can consist of 5 amino acids, 10 amino acids, 15 amino acids, 20 amino acids, or 25 amino acids. In certain embodiments the peptide linker comprises glycines and serines, *e.g.*, (GGGGS)n (SEQ ID NO: 48), where N can be 1, 2, 3, 4, 5, or more. In certain embodiments, the peptide linker consists of GGGGS (SEQ ID NO: 41), GGGGSGGGGS (SEQ ID NO: 42),

GGGGSGGGGSGGGGS (SEQ ID NO: 43), GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 44), or GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 45). In certain embodiments, the heterologous polypeptide can be fused to the N-terminus of the J-chain or fragment or variant thereof, 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. In certain embodiments the heterologous polypeptide can be fused internally within the J-chain. In certain embodiments, the heterologous polypeptide can be a binding domain, *e.g.*, an antigen binding domain. For example, the heterologous polypeptide can be an antibody, a subunit of an antibody, or an antigen-binding fragment of an antibody, *e.g.*, a scFv fragment. In certain embodiments, the binding domain, *e.g.*, scFv fragment can bind to an effector cell, *e.g.*, a T cell or an NK cell. In certain embodiments, the binding domain, *e.g.*, scFv fragment can specifically bind to CD3 on cytotoxic T cells, *e.g.*, to CD3ε. In certain specific embodiments, the modified J-chain of a pentameric IgM-derived binding molecule as provided herein comprises amino acids sequence SEQ ID NO: 36 (V15J) or SEQ ID NO: 37 (V15J*), or a J-chain comprising an anti-CD3ε scFv antigen-binding domain comprising the six complementarity-determining region of murine antibody SP34 (VH = SEQ ID NO: 14, VL = SEQ ID NO: 18), the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 amino acid sequences 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, *e.g.*, the modified J-chain SJ*, comprising the amino acid sequence SEQ ID NO: 39. Other humanized SP35 antibodies include the VH and VL or scFv sequences of A-55 (SEQ ID NOs 22, 23, and 24, respectively, WO2018208864), A-56 (SEQ ID NOs 25, 26, and 27, respectively, WO2018208864), or A-57 (SEQ ID NOs 28, 29, and 30, respectively, WO2018208864), incorporated into the modified J-chains A-55-J* (SEQ ID NO: 31), A-56-J* (SEQ ID NO: 32), and A-57-J* (SEQ ID NO: 33). In certain embodiments a modified J-chain as provided herein can further include an additional heterologous moiety attached, *e.g.*, on the opposite end of the J-chain from the anti-CD3ε scFv binding domain. For example, the modified J-chain can further include the human serum albumin protein. Examples include, but are not limited to, VJH (SEQ ID NO: 34) and VJ*H (SEQ ID NO: 35).

IgM-derived Binding molecules with enhanced serum half-life

**[0166]** A monoclonal population of highly sialylated IgM antibodies, IgM-like antibodies, or IgM-derived binding molecules as provided herein can be further engineered to have enhanced serum half-life. Exemplary IgM heavy chain constant region mutations that can enhance serum half-life of an IgM-derived binding molecule are disclosed in US Patent Application Publication No. US 2020-0239572, which is incorporated by reference herein in its entirety. For example, variant IgM heavy chain constant regions of the population of highly sialylated IgM antibodies, IgM-like antibodies, or IgM-derived binding molecules as provided herein can include an amino acid substitution at an amino acid position corresponding to amino acid S401, E402, E403, R344, and/or E345 of a wild-type human IgM constant region (*e.g.*, SEQ ID NO: 1 or SEQ ID NO: 2). By "an amino acid corresponding to amino acid S401, E402, E403, R344, and/or E345 of a wild-type human IgM constant region" is meant the amino acid in the sequence of the IgM constant region of any species which is homologous to S401, E402, E403, R344, and/or E345 in the human IgM constant region. In certain embodiments, the amino acid corresponding to S401, E402, E403, R344, and/or E345 of SEQ ID NO: 1 or SEQ ID NO: 2 can be substituted with any amino acid, *e.g.*, alanine.

**[0167]** Wild-type J-chains typically include one N-linked glycosylation site. In certain embodiments, a variant J-chain or functional fragment thereof of a pentameric IgM-derived binding molecule as provided herein includes a mutation within the asparagine(N)-linked glycosylation motif N-$X_1$-S/T, *e.g.*, starting at the amino acid position corresponding to amino acid 49 (motif N6) of the mature human J-chain (SEQ ID NO: 6) or J* (SEQ ID NO: 7), wherein N is asparagine, $X_1$ is any amino acid except proline, and S/T is serine or threonine, and wherein the mutation prevents glycosylation at that motif. As demonstrated in US Patent Application Publication No. US 2020-0239572, mutations preventing glycosylation at this site can result in the population of IgM-derived binding molecules, *e.g.*, an IgM antibodies, IgM-like antibodies, or other IgM-derived binding molecules as provided herein, exhibiting 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 mutation or mutations preventing glycosylation in the variant J-chain, and is administered in the same way to the same animal species.

**[0168]** For example, in certain embodiments the variant J-chain or functional fragment thereof of a pentameric IgM-derived binding molecule as provided herein can include an

amino acid substitution at the amino acid position corresponding to amino acid N49 or amino acid S51 SEQ ID NO: 6 or SEQ ID NO: 7, provided that the amino acid corresponding to S51 is not substituted with threonine (T), or wherein the variant J-chain comprises amino acid substitutions at the amino acid positions corresponding to both amino acids N49 and S51 of SEQ ID NO: 6 or SEQ ID NO: 7. In certain embodiments, the position corresponding to N49 of SEQ ID NO: 6 or SEQ ID NO: 7 is substituted with any amino acid, *e.g.*, alanine (A), glycine (G), threonine (T), serine (S) or aspartic acid (D). In a particular embodiment, the position corresponding to N49 of SEQ ID NO: 6 or SEQ ID NO: 7 can be substituted with alanine (A). In another particular embodiment, the position corresponding to N49 of SEQ ID NO: 6 or SEQ ID NO: 7 can be substituted with aspartic acid (D).

Variant Human IgM Constant Regions with Reduced CDC Activity

**[0169]** A monoclonal population of IgM-derived binding molecules, *e.g.*, IgM antibodies, IgM-like antibodies, or other IgM-derived binding molecules as provided herein, can be engineered to exhibit reduced complement-dependent cytotoxicity (CDC) activity to cells in the presence of complement, relative to a reference population of IgM antibodies or IgM-like antibodies with corresponding reference human IgM constant regions identical, except for the mutations conferring reduced CDC activity. These CDC mutations can be combined with any of the mutations to confer increased serum half-life as provided herein. By "corresponding reference human IgM constant region" is meant a human IgM constant region or portion thereof, *e.g.*, a Cμ3 domain, that is identical to the variant IgM constant region except for the modification or modifications in the constant region affecting CDC activity. In certain embodiments, the variant human IgM constant region includes one or more amino acid substitutions, *e.g.*, in the Cμ3 domain, relative to a wild-type human IgM constant region as described, *e.g.*, in PCT Publication No. WO/2018/187702, which is incorporated herein by reference in its entirety. Assays for measuring CDC are well known to those of ordinary skill in the art, and exemplary assays are described *e.g.*, in PCT Publication No. WO/2018/187702.

**[0170]** In certain embodiments, a variant human IgM constant region conferring reduced CDC activity includes an amino acid substitution corresponding to the wild-type human IgM constant region at position P311 of SEQ ID NO: 1 or SEQ ID NO: 2. In other embodiments the variant IgM constant region as provided herein contains an amino acid

substitution corresponding to the wild-type human IgM constant region at position P313 of SEQ ID NO: 1 or SEQ ID NO: 2. In other embodiments the variant IgM constant region as provided herein contains a combination of substitutions corresponding to the wild-type human IgM constant region at positions P311 of SEQ ID NO: 1 or SEQ ID NO: 2 and P313 of SEQ ID NO: 1 or SEQ ID NO: 2. These proline residues can be independently substituted with any amino acid, *e.g.*, with alanine, serine, or glycine. In certain embodiments, a variant human IgM constant region conferring reduced CDC activity includes an amino acid substitution corresponding to the wild-type human IgM constant region at position K315 of SEQ ID NO: 22 or SEQ ID NO: 23. The lysine residue can be independently substituted with any amino acid, *e.g.*, with alanine, serine, glycine, or aspartic acid. In certain embodiments, a variant human IgM constant region conferring reduced CDC activity includes an amino acid substitution corresponding to the wild-type human IgM constant region at position K315 of SEQ ID NO: 22 or SEQ ID NO: 23 with aspartic acid.

Host cells

**[0171]** In certain embodiments, this disclosure provides a host cell that is capable of producing the highly sialylated monoclonal population of binding molecules as provided herein. In certain aspects such a host cell overexpresses ST6GAL1 and/or B4GALT4. The disclosure also provides a method of producing the monoclonal population of binding molecules as provided herein, where the method comprises culturing the provided host cell, and recovering the population of binding molecules.

Methods for producing a highly sialylated population of IgM antibodies, IgM-like antibodies, or IgM-derived binding molecules

**[0172]** This disclosure further provides a method for producing a monoclonal population of highly sialylated multimeric binding molecules as described in detail in this disclosure, where the method includes: providing a cell line that expresses the monoclonal population of binding molecules, culturing the cell line, and recovering the monoclonal population of binding molecules. In certain embodiments, each binding molecule comprises ten or twelve IgM-derived heavy chains, wherein the IgM-derived heavy chains comprise glycosylated IgM heavy chain constant regions, or multimerizing fragments thereof, 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 and wherein an N-linked glycosylation motif comprises the amino acid sequence N-$X_1$-S/T, where N is asparagine, $X_1$ is any amino acid except proline, and S/T is serine or threonine. As provided herein, 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 is/are occupied by complex glycans, and wherein the cell line, culture conditions, recovery process, or a combination thereof is optimized to enrich for complex glycans comprising at least one, two, at least three, or four sialic acid terminal monosaccharides per glycan.

**[0173]** In certain embodiments, the cell line, culture conditions, recovery process, or a combination thereof can be optimized according to the provided method to result in a monoclonal population of binding molecules comprising 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, 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 certain embodiments, the cell line, recovery process, or a combination thereof can be optimized according to the provided method to result in a monoclonal population of binding molecules comprising 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 embodiments, the monoclonal population of binding molecules comprises about 35 to about 40, about 35 to about 45, about 35 to about 50, about 35 to about 55, about 35 to about 60, about 35 to about 65, about 35 to about 70, about 40 to about 45, about 40 to about 50, about 40 to about 55, about 40 to about 60, about 40 to about 65, about 40 to about 70, about 45 to about 50, about 45 to about 55, about 45 to about 60, about 45 to about 65, about 45 to about 70, about 50 to about 55, about 50 to about 60, about 50 to about 65, about 50 to about 70, about 55 to about 60, about 55 to about 65, about 55 to about 70, about 60 to about 65, about 60 to about 70, or about 65 to about 70 moles sialic acid per mole of binding molecule. In some embodiments, the monoclonal population of binding molecules comprises about 40 to about 55 moles sialic acid per mole of binding molecule. According to the provided method, the IgM heavy chain constant regions can be derived from human IgM heavy chain constant regions comprising five N-linked glycosylation motifs N-$X_1$-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 certain embodiments, on average one, two, or all three of motifs N1, N2, and N3 in the population of binding molecules are occupied by complex glycans that can be sialylated by the provided method.

**[0174]** In certain embodiments, the cell line cultured according to provided method is modified to overexpress a sialyltransferase. In certain embodiments, the overexpressed sialyltransferase is a 2,6-sialyl transferase. In certain embodiments, the overexpressed sialyltransferase is human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1). In certain embodiments, the overexpressed sialyltransferase is a 2,3-sialyl transferase. The cell line cultured according to provided method can also be modified to overexpress a galactosyltransferase. In certain embodiments the overexpressed galactosyltransferase is human beta-1,4-galactosyltransferase 4 (B4GALT4). The cell line cultured according to provided method can also be modified to overexpress an UDP-GlcNAc 2-Epimerase/ManNAc Kinase enzyme (GNE), such as GNE comprising an R263 or R266 mutation, such as a Q, W, or L mutation; an α-mannosidase II; an N-Acetylglucosaminyltransferase-II (GNT-II); an N-Acetylglucosaminyltransferase-IV (GNT-IV); an N-Acetylglucosaminyltransferase-V (GNT-V); a CMP-sialic acid synthase (CMP-SAS), a CMP-sialic acid transporter (CMP-SAT), or any combination thereof. In certain embodiments the cell line cultured according to provided method can also be modified to block expression of certain sialidases. In certain embodiments the cell line cultured according to provided method can also be modified to block expression of a neuraminidase.

**[0175]** In certain embodiments of the provided method, the recovery process includes subjecting the monoclonal population of multimeric binding molecules to glycoengineering during downstream processing, to produce, *e.g.*, a monoclonal population of glycoengineered IgM antibodies, IgM-like antibodies, or IgM-derived binding molecules, or "GEMs." In certain embodiments the GEMs are highly sialylated, *e.g.*, possessing at least 35 moles sialic acid per mole of binding molecule. Production of GEMs is described in detail elsewhere herein, and any and all aspects of the production of GEMs can be included in the provided method. In certain embodiments, the production of GEMs includes contacting the monoclonal population of binding molecules with a soluble sialyltransferase and a sialic acid substrate. In certain embodiments, the soluble sialyltransferase can be a soluble variant of human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) (SEQ ID NO: 3). In certain embodiments the soluble

variant of ST6GAL1 includes amino acids x to 406 of SEQ ID NO: 3, wherein x is an integer from 27 to 120. For example, the soluble variant of ST6GAL1 can include 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 certain embodiments, the sialic acid substrate can include cytidine monophosphate (CMP)-N-acetyl-neuraminic acid (CMP-NANA), or a derivative thereof.

**[0176]** As described elsewhere herein, the inventors have discovered that the production of highly sialylated IgM antibodies, IgM-like antibodies, or IgM-derived binding molecules requires much less enzyme than a comparable method for sialylating IgG. For example, the mass ratio of binding molecule: sialyltransferase can be about 80:1 to about 5000:1. In some embodiments, the mass ratio of binding molecule: sialyltransferase can be about 80:1 to about 100:1, about 80:1 to about 250:1, about 80:1 to about 500:1, about 80:1 to about 750:1, about 80:1 to about 1000:1, about 80:1 to about 1250:1, about 80:1 to about 1500:1, about 80:1 to about 1750:1, about 80:1 to about 2000:1, about 80:1 to about 2500:1, about 80:1 to about 3000:1, about 80:1 to about 3500:1, about 80:1 to about 4000:1, about 80:1 to about 4500:1, about 80:1 to about 5000:1, about 250:1 to about 500:1, about 250:1 to about 750:1, about 250:1 to about 1000:1, about 250:1 to about 1250:1, about 250:1 to about 1500:1, about 250:1 to about 1750:1, about 250:1 to about 2000:1, about 250:1 to about 2500:1, about 250:1 to about 3000:1, about 250:1 to about 3500:1, about 250:1 to about 4000:1, about 250:1 to about 4500:1, about 250:1 to about 5000:1, about 500:1 to about 750:1, about 500:1 to about 1000:1, about 500:1 to about 1250:1, about 500:1 to about 1500:1, about 500:1 to about 1750:1, about 500:1 to about 2000:1, about 500:1 to about 2500:1, about 500:1 to about 3000:1, about 500:1 to about 3500:1, about 500:1 to about 4000:1, about 500:1 to about 4500:1, about 500:1 to about 5000:1, about 1000:1 to about 1250:1, about 1000:1 to about 1500:1, about 1000:1 to about 1750:1, about 1000:1 to about 2000:1, about 1000:1 to about 2500:1, about 1000:1 to about 3000:1, about 1000:1 to about 3500:1, about 1000:1 to about 4000:1, about 1000:1 to about 4500:1, about 1000:1 to about 5000:1, about 1500:1 to about 1750:1, about 1500:1 to about 2000:1, about 1500:1 to about 2500:1, about 1500:1 to about 3000:1, about 1500:1 to about 3500:1, about 1500:1 to about 4000:1, about 1500:1 to about 4500:1, about 1500:1 to about 5000:1, about 2000:1 to about 2500:1, about 2000:1 to about 3000:1, about 2000:1 to about 3500:1, about 2000:1

to about 4000:1, about 2000:1 to about 4500:1, about 2000:1 to about 5000:1, about 2500:1 to about 3000:1, about 2500:1 to about 3500:1, about 2500:1 to about 4000:1, about 2500:1 to about 4500:1, about 2500:1 to about 5000:1, about 3000:1 to about 3500:1, about 3000:1 to about 4000:1, about 3000:1 to about 4500:1, about 3000:1 to about 5000:1, about 3500:1 to about 4000:1, about 3500:1 to about 4500:1, about 3500:1 to about 5000:1, about 4000:1 to about 4500:1, or about 4000:1 to about 5000:1. In certain embodiments, the molar ratio of binding molecule: sialyltransferase can be about 200:1, 175:1, 150:1, 155:1, 140:1, 135:1, 130:1, 125:1, 120:1, 115:1, 110:1, 105:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, or 50:1. For example, the mass ratio of binding molecule: sialic acid substrate: sialyltransferase can be about 2000:500:1. In certain embodiments, the molar ratio of binding molecule: sialyltransferase can be about 200:1, 175:1, 150:1, 155:1, 140:1, 135:1, 130:1, 125:1, 120:1, 115:1, 110:1, 105:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, or 50:1. In certain embodiments the molar ratio of binding molecule to sialyltransferase can be about 80:1. As mentioned elsewhere herein, production of GEMs can further include contacting the monoclonal population of binding molecules with a galactosyltransferase, *e.g.*, a soluble variant of human beta-1,4-galactosyltransferase 4 (B4GALT4) (SEQ ID NO: 4) and a galactose substrate, *e.g.*, uridine-diphosphate-α-D-galactose (UDP-Gal). The contacting with galactosyltransferase and galactose substrate can occur prior to or simultaneously with the contacting with the soluble sialyltransferase and sialic acid substrate.

**[0177]** In some embodiments, the mass ratio of sialic acid substrate: sialyltransferase can be about 5:1 to about 3000:1, such as about 5:1 to about 10:1, about 5:1 to about 50:1, about 5:1 to about 100:1, about 5:1 to about 500:1, about 5:1 to about 1000:1, about 5:1 to about 1500:1, about 5:1 to about 2000:1, about 5:1 to about 2500:1, about 10:1 to about 50:1, about 10:1 to about 100:1, about 10:1 to about 500:1, about 10:1 to about 1000:1, about 10:1 to about 1500:1, about 10:1 to about 2000:1, about 10:1 to about 2500:1, about 10:1 to about 3000:1, about 50:1 to about 100:1, about 50:1 to about 500:1, about 50:1 to about 1000:1, about 50:1 to about 1500:1, about 50:1 to about 2000:1, about 50:1 to about 2500:1, about 50:1 to about 3000:1, about 100:1 to about 500:1, about 100:1 to about 1000:1, about 100:1 to about 1500:1, about 100:1 to about 2000:1, about 100:1 to about 2500:1, about 100:1 to about 3000:1, about 500:1 to about 1000:1, about 500:1 to about 1500:1, about 500:1 to about 2000:1, about 500:1 to about 2500:1, about 500:1 to about 3000:1, about 1000:1 to about 1500:1, about 1000:1 to about 2000:1, about 1000:1 to about

2500:1, about 1000:1 to about 3000:1, about 1500:1 to about 2000:1, about 1500:1 to about 2500:1, about 1500:1 to about 3000:1, about 2000:1 to about 2500:1, about 2000:1 to about 3000:1, or about 2500:1 to about 3000:1.

**[0178]** In some embodiments, the mass ratio of antibody: sialic acid substrate can be about 1:1 to about 40:1, such as about 1:1 to about 2:1, about 1:1 to about 4:1, about 1:1 to about 6:1, about 1:1 to about 8:1, about 1:1 to about 10:1, about 1:1 to about 15:1, about 1:1 to about 20:1, about 2:1 to about 4:1, about 2:1 to about 6:1, about 2:1 to about 8:1, about 2:1 to about 10:1, about 2:1 to about 15:1, about 2:1 to about 20:1, about 2:1 to about 40:1, about 4:1 to about 6:1, about 4:1 to about 8:1, about 4:1 to about 10:1, about 4:1 to about 15:1, about 4:1 to about 20:1, about 4:1 to about 40:1, about 6:1 to about 8:1, about 6:1 to about 10:1, about 6:1 to about 15:1, about 6:1 to about 20:1, about 6:1 to about 40:1, about 8:1 to about 10:1, about 8:1 to about 15:1, about 8:1 to about 20:1, about 8:1 to about 40:1, about 10:1 to about 15:1, about 10:1 to about 20:1, about 10:1 to about 40:1, about 15:1 to about 20:1, about 15:1 to about 40:1, or about 20:1 to about 40:1.

**[0179]** In some embodiments, the method comprises contacting the monoclonal population of binding molecules with a soluble sialyltransferase and a sialic acid substrate for at least 30 minutes, such as at least 45 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 10 hours, at least 12 hours, at least 18 hours, at least 24 hours, at least 36 hours, or at least 48 hours. In some embodiments, the contacting occurs for about 30 minutes to about 48 hours, such as about 30 minutes to about 4 hours, about 30 minutes to about 5 hours, about 30 minutes to about 6 hours, about 30 minutes to about 7 hours, about 30 minutes to about 10 hours, about 30 minutes to about 12 hours, about 30 minutes to about 18 hours, about 30 minutes to about 24 hours, about 30 minutes to about 36 hours, about 2 hours to about 48 hours, about 3 hours to about 6 hours, about 3 hours to about 10 hours, about 3 hours to about 12 hours, about 3 hours to about 18 hours, about 3 hours to about 24 hours, about 3 hours to about 36 hours, about 3 hours to about 48 hours, about 4 hours to about 10 hours, about 4 hours to about 12 hours, about 4 hours to about 18 hours, about 4 hours to about 24 hours, about 4 hours to about 36 hours, about 4 hours to about 48 hours, about 5 hours to about 10 hours, about 5 hours to about 12 hours, about 5 hours to about 18 hours, about 5 hours to about 24 hours, about 5 hours to about 36 hours, about 5 hours to about 48 hours, about 7 hours to about 10 hours, about 7 hours to about 12 hours, about 7 hours to about 18 hours, about 7 hours to about 24 hours, about 7 hours to about 36 hours, about 7 hours to about

48 hours, about 10 hours to about 18 hours, about 10 hours to about 24 hours, about 10 hours to about 36 hours, about 10 hours to about 48 hours, about 12 to about 18 hours, about 12 hours to about 24 hours, about 12 hours to about 36 hours, about 12 hours to about 48 hours, about 18 hours to about 24 hours, about 18 hours to about 36 hours, about 18 hours to about 48 hours, about 24 hours to about 36 hours, about 24 hours to about 48 hours, or about 36 hours to about 48 hours,

**[0180]** In some embodiments, the method comprises contacting the monoclonal population of binding molecules with a soluble sialyltransferase and a sialic acid substrate at a temperature at about 2 °C to about 40 °C, such as at about 2 °C to about 37 °C, 2 °C to about 30 °C, 2 °C to about 25 °C, 2 °C to about 22 °C, 2 °C to about 20 °C, 2 °C to about 10 °C, about 4 °C to about 40 °C, about 4 °C to about 37 °C, 4 °C to about 30 °C, 4 °C to about 25 °C, 4 °C to about 22 °C, 4 °C to about 20 °C, 4 °C to about 10 °C, about 10 °C to about 40 °C, about 10 °C to about 37 °C, 10 °C to about 30 °C, 10 °C to about 25 °C, 10 °C to about 22 °C, 10 °C to about 20 °C, about 20 °C to about 40 °C, about 20 °C to about 37 °C, 20 °C to about 30 °C, 20 °C to about 25 °C, 20 °C to about 22 °C, about 22 °C to about 40 °C, about 22 °C to about 37 °C, 22 °C to about 30 °C, 22 °C to about 25 °C, about 25 °C to about 40 °C, about 25 °C to about 37 °C, 25 °C to about 30 °C, about 30 °C to about 40 °C, or about 30 °C to about 37 °C.

**[0181]** This disclosure employs, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Green and Sambrook, ed. (2012) Molecular Cloning A Laboratory Manual (4th ed.; Cold Spring Harbor Laboratory Press); Sambrook *et al.*, ed. (1992) Molecular Cloning: A Laboratory Manual, (Cold Springs Harbor Laboratory, NY); D. N. Glover and B.D. Hames, eds., (1995) DNA Cloning 2d Edition (IRL Press), Volumes 1-4; Gait, ed. (1990) Oligonucleotide Synthesis (IRL Press); Mullis *et al.* U.S. Pat. No. 4,683,195; Hames and Higgins, eds. (1985) Nucleic Acid Hybridization (IRL Press); Hames and Higgins, eds. (1984) Transcription And Translation (IRL Press); Freshney (2016) Culture Of Animal Cells, 7th Edition (Wiley-Blackwell); Woodward, J., Immobilized Cells And Enzymes (IRL Press) (1985); Perbal (1988) A Practical Guide To Molecular Cloning; 2d Edition (Wiley-Interscience); Miller and Calos eds. (1987) Gene Transfer Vectors For Mammalian Cells, (Cold Spring Harbor Laboratory); S.C. Makrides (2003) Gene Transfer and Expression in Mammalian Cells (Elsevier Science); Methods in

Enzymology, Vols. 151-155 (Academic Press, Inc., N.Y.); Mayer and Walker, eds. (1987) Immunochemical Methods in Cell and Molecular Biology (Academic Press, London); Weir and Blackwell, eds.; and in Ausubel *et al.* (1995) Current Protocols in Molecular Biology (John Wiley and Sons).

**[0182]** General principles of antibody engineering are set forth, *e.g.*, in Strohl, W.R., and L.M. Strohl (2012), Therapeutic Antibody Engineering (Woodhead Publishing). General principles of protein engineering are set forth, *e.g.*, in Park and Cochran, eds. (2009), Protein Engineering and Design (CDC Press). General principles of immunology are set forth, *e.g.*, in: Abbas and Lichtman (2017) Cellular and Molecular Immunology 9th Edition (Elsevier). Additionally, standard methods in immunology known in the art can be followed, *e.g.*, in Current Protocols in Immunology (Wiley Online Library); Wild, D. (2013), The Immunoassay Handbook 4th Edition (Elsevier Science); Greenfield, ed. (2013), Antibodies, a Laboratory Manual, 2d Edition (Cold Spring Harbor Press); and Ossipow and Fischer, eds., (2014), Monoclonal Antibodies: Methods and Protocols (Humana Press).

**[0183]** All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.

## Exemplary Embodiments

**[0184]** Among the provided embodiments are:

**[0185]** Embodiment 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) moles sialic acid per mole of the binding molecules.

**[0186]** Embodiment 2. The monoclonal population of binding molecules of embodiment 1, comprising 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.

**[0187]** Embodiment 3. The monoclonal population of binding molecules of embodiment 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.

**[0188]** Embodiment 4. The monoclonal population of binding molecules of any one of embodiments 1 to 3, 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 (allele IGHM*03) or SEQ ID NO: 2 (allele IGHM*04).

**[0189]** Embodiment 5. The monoclonal population of binding molecules of embodiment 4, wherein motifs N1, N2, and N3 are occupied by complex glycans.

**[0190]** Embodiment 6. The monoclonal population of binding molecules of any one of embodiments 1 to 5, produced by the method of cell line modification, in vitro glycoengineering, or any combination thereof.

**[0191]** Embodiment 7. The monoclonal population of binding molecules of embodiment 6, 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.

**[0192]** Embodiment 8. The monoclonal population of binding molecules of embodiment 7, wherein the sialyltransferase comprises human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) (SEQ ID NO: 3).

**[0193]** Embodiment 9. The monoclonal population of binding molecules of embodiment 7 or embodiment 8, wherein the cell line modification further comprises 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.

**[0194]** Embodiment 10. The monoclonal population of binding molecules of embodiment 9, wherein the galactosyltransferase comprises human beta-1,4-galactosyltransferase 4 (B4GALT4) (SEQ ID NO: 4).

**[0195]** Embodiment 11. The monoclonal population of binding molecules of any one of embodiments 6 to 10, wherein in vitro glycoengineering comprises contacting the monoclonal population of binding molecules with a soluble sialyltransferase and a sialic acid substrate.

**[0196]** Embodiment 12. The monoclonal population of binding molecules of embodiment 11, wherein the sialyltransferase comprises a soluble variant of human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) (SEQ ID NO: 3).

**[0197]** Embodiment 13. The monoclonal population of binding molecules of embodiment 12, wherein the soluble variant of ST6GAL1 comprises amino acids x to 406 of SEQ ID NO: 3, wherein x is an integer from 27 to 120.

**[0198]** Embodiment 14. The monoclonal population of binding molecules of embodiment 13, wherein the soluble variant of ST6GAL1 comprises 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.

**[0199]** Embodiment 15. The monoclonal population of binding molecules of any one of embodiments 11 to 14, wherein the sialic acid substrate comprises cytidine monophosphate-N-acetyl-neuraminic acid (CMP-NANA).

**[0200]** Embodiment 16. The monoclonal population of binding molecules of any one of embodiments 11 to 15, wherein the mass ratio of binding molecule: sialic acid substrate is about 1:4 to about 40:1.

**[0201]** Embodiment 17. The monoclonal population of binding molecules of any one of embodiments 11 to 16, wherein the mass ratio of binding molecule: sialyltransferase is about 80:1 to about 5000:1.

**[0202]** Embodiment 18. The monoclonal population of binding molecules of any one of embodiments 11 to 17, wherein the mass ratio of binding molecule: sialyltransferase is about 2000:1.

**[0203]** Embodiment 19. The monoclonal population of binding molecules of embodiment 18, wherein the mass ratio of binding molecule: sialic acid substrate: sialyltransferase is about 2000:500:1.

**[0204]** Embodiment 20. The monoclonal population of binding molecules of any one of embodiments 11 to 17, wherein the molar ratio of binding molecule: sialyltransferase is about 80:1.

**[0205]** Embodiment 21. The monoclonal population of binding molecules of embodiment 20, wherein the molar ratio of binding molecule: sialic acid substrate: sialyltransferase is about 80:500:1.

**[0206]** Embodiment 22. The monoclonal population of binding molecules of any one of embodiments 11 to 17, wherein the mass ratio of binding molecule: sialyltransferase is about 500:1.

**[0207]** Embodiment 23. The monoclonal population of binding molecules of embodiment 22, wherein the mass ratio of binding molecule: sialic acid substrate: sialyltransferase is about 500:62.5:1.

**[0208]** Embodiment 24. The monoclonal population of binding molecules of any one of embodiments 11 to 23, wherein 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.

**[0209]** Embodiment 25. The monoclonal population of binding molecules of embodiment 24, wherein 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.

**[0210]** Embodiment 26. The monoclonal population of binding molecules of any one of embodiments 11 to 25, wherein 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.

**[0211]** Embodiment 27. The monoclonal population of binding molecules of embodiment 26, wherein the contacting occurs at 15° C to about 37° C, 15° C to about 30° C, or 15° C to about 25° C.

**[0212]** Embodiment 28. The monoclonal population of binding molecules of any one of embodiments 11 to 27, wherein in vitro glycoengineering further comprises contacting the monoclonal population of binding molecules with a galactosyltransferase and a galactose substrate.

**[0213]** Embodiment 29. The monoclonal population of binding molecules of embodiment 28, wherein the galactosyltransferase comprises a soluble variant of human beta-1,4-galactosyltransferase 4 (B4GALT4) (SEQ ID NO: 4).

**[0214]** Embodiment 30. The monoclonal population of binding molecules of embodiment 29, wherein the soluble variant of B4GALT4 comprises amino acids x to 344 of SEQ ID NO: 4, wherein x is an integer from 39 to 120.

**[0215]** Embodiment 31. The monoclonal population of binding molecules of embodiment 30, wherein the soluble variant of B4GALT4 comprises 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.

**[0216]** Embodiment 32. The monoclonal population of binding molecules of any one of embodiments 28 to 31, wherein the galactose substrate comprises uridine-diphosphate-α-D-galactose (UDP-Gal).

**[0217]** Embodiment 33. The monoclonal population of binding molecules of any one of embodiments 28 to 32, wherein 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.

**[0218]** Embodiment 34. The monoclonal population of binding molecules of any one of embodiments 1 to 33, wherein each binding molecule is multispecific, and wherein two or more binding domains associated with the IgM heavy chain constant regions of each binding molecule specifically bind to different targets.

**[0219]** Embodiment 35. The monoclonal population of binding molecules of any one of embodiments 1 to 33, wherein the binding domains associated with the IgM heavy chain constant regions of each binding molecule specifically bind to the same target.

**[0220]** Embodiment 36. The monoclonal population of binding molecules of embodiment 35, wherein the binding domains associated with the IgM heavy chain constant regions of each binding molecule are identical.

**[0221]** Embodiment 37. The monoclonal population of binding molecules of any one of embodiments 34 to 36, wherein the binding domains are antibody-derived antigen-binding domains.

**[0222]** Embodiment 38. The monoclonal population of binding molecules of embodiment 37, 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.

**[0223]** Embodiment 39. The monoclonal population of binding molecules of embodiment 38, wherein each antigen-binding domain of each binding molecule binds to the same target.

**[0224]** Embodiment 40. The monoclonal population of binding molecules of embodiment 39, wherein each antigen-binding domain of each binding molecule is identical.

**[0225]** Embodiment 41. The monoclonal population of binding molecules of any one of embodiments 1 to 40, wherein the target is a target epitope, a target antigen, a target cell, a target organ, or a target virus.

**[0226]** Embodiment 42. The monoclonal population of binding molecules of any one of embodiments 1 to 41, wherein each binding molecule is pentameric and further comprises a J-chain, or functional fragment thereof, or a functional variant thereof.

**[0227]** Embodiment 43. The monoclonal population of binding molecules of embodiment 42, 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.

**[0228]** Embodiment 44. The monoclonal population of binding molecules of embodiment 43, wherein the J-chain comprises 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).

**[0229]** Embodiment 45. The monoclonal population of binding molecules of any one of embodiments 42 to 44, wherein the J-chain is a functional variant J-chain comprising 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 wherein 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.

**[0230]** Embodiment 46. The monoclonal population of binding molecules of embodiment 45, wherein the variant J-chain or functional fragment thereof comprises one, two, three, or four single amino acid substitutions, deletions, or insertions relative to the reference J-chain.

**[0231]** Embodiment 47. The monoclonal population of binding molecules of embodiment 45 or embodiment 46, 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.

**[0232]** Embodiment 48. The monoclonal population of binding molecules of embodiment 47, wherein the amino acid corresponding to Y102 of SEQ ID NO: 6 is substituted with alanine (A).

**[0233]** Embodiment 49. The monoclonal population of binding molecules of embodiment 48, wherein the J-chain comprises the amino acid sequence SEQ ID NO: 7.

**[0234]** Embodiment 50. The monoclonal population of binding molecules of any one of embodiments 42 to 49, 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.

**[0235]** Embodiment 51. The monoclonal population of binding molecules of embodiment 50, wherein the heterologous moiety is a polypeptide fused to the J-chain or fragment or variant thereof.

**[0236]** Embodiment 52. The monoclonal population of binding molecules of embodiment 51, wherein the heterologous polypeptide is fused to the J-chain or fragment or variant thereof via a peptide linker.

**[0237]** Embodiment 53. The monoclonal population of binding molecules of embodiment 52, wherein the peptide linker comprises at least 5 amino acids, but no more than 25 amino acids.

**[0238]** Embodiment 54. The monoclonal population of binding molecules of embodiment 52 or embodiment 53, wherein the peptide linker consists of GGGGSGGGGSGGGGS (SEQ ID NO: 43).

**[0239]** Embodiment 55. The monoclonal population of binding molecules of any one of embodiments 51 to 54, wherein 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.

**[0240]** Embodiment 56. The monoclonal population of binding molecules of any one of embodiments 51 to 55, wherein 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.

**[0241]** Embodiment 57. The monoclonal population of binding molecules of any one of embodiments 51 to 56, wherein the heterologous polypeptide comprises a binding domain.

**[0242]** Embodiment 58. The monoclonal population of binding molecules of embodiment 57, wherein the binding domain of the heterologous polypeptide is an antibody or antigen-binding fragment thereof.

**[0243]** Embodiment 59. The monoclonal population of binding molecules of embodiment 58, wherein the antigen-binding fragment is a scFv fragment.

**[0244]** Embodiment 60. The monoclonal population of binding molecules of embodiment 59, wherein the heterologous scFv fragment binds to CD3ε.

**[0245]** Embodiment 61. The monoclonal population of binding molecules of embodiment 60, wherein the modified J-chain comprises 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-CD3ε scFv comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 amino acid sequences comprising 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.

**[0246]** Embodiment 62. A pharmaceutical composition comprising the monoclonal population of binding molecules of any one of embodiments 1 to 61 and a pharmaceutically acceptable excipient.

**[0247]** Embodiment 63. A recombinant host cell that produces the monoclonal population of binding molecules of any one of embodiments 1 to 61.

**[0248]** Embodiment 64. A method of producing the monoclonal population of binding molecules of any one of embodiments 1 to 61, comprising culturing the host cell of embodiment 62, and recovering the population of binding molecules.

**[0249]** Embodiment 65. 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.

**[0250]** Embodiment 66. The method of embodiment 65, 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.

**[0251]** Embodiment 67. The method of embodiment 66, wherein 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.

**[0252]** Embodiment 68. The method of any one of embodiments 65 to 67, 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).

**[0253]** Embodiment 69. The method of embodiment 68, wherein on average one, two, or all three of motifs N1, N2, and N3 in the population of binding molecules are occupied by complex glycans.

**[0254]** Embodiment 70. The method of any one of embodiments 65 to 69, wherein the provided cell line is modified to overexpress a sialyltransferase.

[0255] Embodiment 71. The method of embodiment 70, wherein the sialyltransferase comprises human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1, SEQ ID NO: 3).

[0256] Embodiment 72. The method of any one of embodiments 65 to 71, wherein the recovery process comprises subjecting the monoclonal population of binding molecules to in vitro glycoengineering.

[0257] Embodiment 73. The method of embodiment 72, wherein the in vitro glycoengineering comprises contacting the monoclonal population of binding molecules with a soluble sialyltransferase and a sialic acid substrate.

[0258] Embodiment 74. The method of embodiment 73, wherein the sialyltransferase comprises a soluble variant of human beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) (SEQ ID NO: 3).

[0259] Embodiment 75. The method of embodiment 74, wherein the soluble variant of ST6GAL1 comprises amino acids x to 406 of SEQ ID NO: 3, wherein x is an integer from 27 to 120.

[0260] Embodiment 76. The method of embodiment 75, wherein the soluble variant of ST6GAL1 comprises 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.

[0261] Embodiment 77. The method of any one of embodiments 73 to 75, wherein the sialic acid substrate comprises cytidine monophosphate (CMP)-N-acetyl-neuraminic acid (CMP-NANA).

[0262] Embodiment 78. The method of any one of embodiments 73 to 77, wherein the mass ratio of binding molecule: sialic acid substrate is about 1:4 to about 40:1.

[0263] Embodiment 79. The method of any one of embodiments 73 to 78, wherein the mass ratio of binding molecule: sialyltransferase is about 80:1 to about 10000:1.

[0264] Embodiment 80. The method of any one of embodiments 73 to 79, wherein the mass ratio of binding molecule: sialyltransferase is about 2000:1.

[0265] Embodiment 81. The method of embodiment 80, wherein the mass ratio of binding molecule: sialic acid substrate: sialyltransferase is about 2000:500:1.

**[0266]** Embodiment 82. The method of any one of embodiments 73 to 79, wherein the molar ratio of binding molecule: sialyltransferase is about 80:1.

**[0267]** Embodiment 83. The method of embodiment 82, wherein the molar ratio of binding molecule: sialic acid substrate: sialyltransferase is about 80:500:1.

**[0268]** Embodiment 84. The method of any one of embodiments 73 to 79, wherein the mass ratio of binding molecule: sialyltransferase is about 500:1.

**[0269]** Embodiment 85. The method of embodiment 84, wherein the mass ratio of binding molecule: sialic acid substrate: sialyltransferase is about 500:62.5:1.

**[0270]** Embodiment 86. The method of any one of embodiments 73 to 85, wherein 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.

**[0271]** Embodiment 87. The method of embodiment 86, wherein 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.

**[0272]** Embodiment 88. The method of any one of embodiments 73 to 87, wherein 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.

**[0273]** Embodiment 89. The method of embodiment 88, wherein the contacting occurs at 15° C to about 37° C, 15° C to about 30° C, or 15° C to about 25° C.

**[0274]** Embodiment 90. The method of any one of embodiments 73 to 77, wherein in vitro glycoengineering further comprises contacting the monoclonal population of binding molecules with a galactosyltransferase and a galactose substrate.

**[0275]** Embodiment 91. The method of embodiment 90, wherein the galactosyltransferase comprises a soluble variant of human beta-1,4-galactosyltransferase 4 (B4GALT4) (SEQ ID NO: 4).

**[0276]** Embodiment 92. The method of embodiment 90 or embodiment 91, wherein the galactose substrate comprises uridine-diphosphate-α-D-galactose (UDP-Gal).

**[0277]** Embodiment 93. The method of any one of embodiments 90 to 92, wherein 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.

**[0278]** The following examples are offered by way of illustration and not by way of limitation.

# Examples

## Example 1: Materials and Methods

### Population of IgM antibodies

**[0279]** Except as noted below, these experiments were carried out on a monoclonal population of the IgM bispecific antibody CD20 x CD3 IGM-A, which includes IgM heavy chains comprising wild-type human IgM constant regions (*e.g.*, SEQ ID NO: 1 or SEQ ID NO: 2) and an anti-CD20 VH region of SEQ ID NO: 8, light chains comprising the antiCD20 VL region of SEQ ID NO: 9, and a modified J-chain that binds to CD3 comprising amino acids 20-420 of SEQ ID NO: 34. CD20 x CD3 IGM-A is described in detail in U.S. Patent Application Publication No. US-2018-0265596-A1, which is incorporated herein by reference in its entirety. Glycoengineered IgM antibodies, IgM-like antibodies, or IgM-derived binding molecules are referred to as "GEMs" throughout the Examples regardless of the method of glycoengineering.

### Glycoengineering of IgM antibody populations

**[0280]** Along with various controls, varying amounts of a truncated version of human α-2,6 sialyltransferase as indicated in the Examples below ("truncated human ST6," available from Roche Diagnostics, Inc. (Material Number 07012250103 or Material Number 08098174103)) or from Agilent (Part Number GKT-S26) was added to a 20 µl reaction solution that contained a partially purified monoclonal population of an IgM antibody, *e.g.*, anti-CD20 x CD3 IGM-A, and varying amounts of cytidine-5′-monophospho-N-acetylneuraminic acid sodium salt (CMP-NANA), dissolved in 50 mM Tris-acetate (pH 7.5). The reactions were allowed to run for 8 hours at 37 °C unless indicated otherwise. Reactions were stopped by freezing at –20 °C. The ST6 treated IgM populations were further purified, *e.g.*, by anion exchange chromatography and/or mixed mode chromatography, prior to further analyses.

### Total Sialic Acid Quantitation

**[0281]** The Sialic Acid (NANA) Assay Kit (Agilent AdvanceBio Total Sialic Acid Quantitation Kit) measures free or released sialic acid (N-acetylneuraminic acid (NANA)) from glycoproteins. The assay uses an enzyme coupled reaction in which oxidation of free sialic acid creates an intermediate that reacts stoichiometrically with the probe to generate

a product that can be detected by absorbance (OD = 530 nm) or fluorescence (excitation/emission (Ex/Em) =530/590 nm). The kit measures sialic acid in the linear range of 40 pmol-1,000 pmol with a detection sensitivity of 0.15 mg/ml concentration for IgM antibodies. The kit was used according to manufacturers' recommendations. Briefly, samples were digested with sialidase A for 2 hours. A Bovine Fetuin Control protein was used as a positive control with an expected range of 9.6-13.9 mol/mol. Sialic acid standards were prepared with the following pmol for fluorescent measurement: 1,000, 500, 250 and 0 pmol. Conversion and Developer mix was then prepared according to the Table 2 below.

Table 2: Sialic Acid Quantitation Assay

| Number of Wells | Conversion Reagent Solution (µL) | HRP Solution (µL) | SAQ Buffer C (µL) | SAQ Dye (µL) | Total (µL) | Number of Wells |
|---|---|---|---|---|---|---|
| n | 30/sample | 15/sample | 5/sample | 5/sample | 55 | N |
| 16 | 480 | 240 | 80 | 80 | 880 | 16 |
| 36 | 1080 | 540 | 180 | 180 | 1980 | 36 |
| 48 | 1440 | 720 | 240 | 240 | 2640 | 48 |

**[0282]** Once sialic acid is released by the sialidase A digestion, N-acetylneuraminic aldolase catalyzes the reaction to form pyruvic acid. The reaction then goes through an additional step with pyruvate oxidase as the catalyst to form hydrogen peroxide which forms a 1:1 complex with the dye to form a fluorescence reporter dye. This dye can be read by fluorescence detection (Ex/Em=530/590 nm) which is then correlated to give a sialic acid level mol/mol from the sialic acid standard curve.

Example 2: Effect of α-2,6 sialyltransferase concentration on sialylation of IgM antibody populations

**[0283]** Varying amounts of truncated human ST6 were added to a 20 µl reaction solution comprising 60 µg of anti-CD20 x CD3 IGM-A (3 mg/ml) and 30 µg of cytidine-5′-monophospho-N-acetylneuraminic acid sodium salt (CMP-NANA, 1.5 mg/ml) as described in Example 1. The resulting sialylation was quantitated as described in Example 1, and the amounts of sialylation compared to truncated human ST6 concentration are shown in **FIG. 4**.

**[0284]** These results demonstrate that ST6 concentrations as low as 1.5 µg/ml (molar ratio of IgM to truncated human ST6 of 80:1) can be used to generate SA level of 40 mol/mol,

and as the truncated human ST6 concentration is increased to 30 μg/ml or higher (molar ratio of IgM to truncated human ST6 of about 4:1 or higher), SA levels greater than 60 mol/mol can be generated.

Example 3: Sialylation of other IgM antibodies

**[0285]** To determine if the *in vitro* sialylation procedures developed above could be applied to other IgM antibodies, two other CHO cell lines expressing recombinant IgM antibodies, pentameric anti-DR5 IGM-B (VH: SEQ ID NO: 10, VL: SEQ ID NO: 11, see U.S. Patent No. 7,521,048) and hexameric anti-DR5 IGM-C (VH: SEQ ID NO: 12, VL: SEQ ID NO: 13, see U.S. Patent No. 7,790,165), were sialylated and analyzed as described Example 1. The 20 μl reactions included 0.28 μg truncated human ST6 (final concentration of 14 μg/mL), 60 μg anti-DR5 IGM-B or anti-DR5 IGM-C (for an approximately 8:1 IgM to truncated human ST6 molar ratio), and 30 μg CMP-NANA were used. Control reactions without the truncated human ST6 were also run. The resulting sialylation was determined as described in Example 1 and the amounts of sialylation for each condition are shown in **FIG. 5** and Table 3.

Table 3: Sialylation of Anti-DR5 Antibodies

| Antibody | Condition | SA (mol/mol) |
|---|---|---|
| Anti-DR5 IGM-B | No ST6 | 16 |
| Anti-DR5 IGM-B-GEM | + ST6 | 52 |
| Anti-DR5 IGM-C | No ST6 | 9 |
| Anti-DR5 IGM-C-GEM | + ST6 | 52 |

Example 4: Sialylation level of human serum IgM

**[0286]** The sialylation level of human serum IgM (obtained from Sigma, cat#I8260-25 mg) was determined using the Sialic Acid (NANA) Assay Kit (Agilent AdvanceBio Total Sialic Acid Quantitation Kit) as explained in Example 1.

**[0287]** Once sialic acid is released by the sialidase A digest, N-acetylneuraminic aldolase catalyzes the reaction to form pyruvic acid. The reaction then goes through an additional step with pyruvate oxidase as the catalyst forming hydrogen peroxide which forms a 1:1 complex with the dye to form a fluorescence reporter dye. This dye can be read by fluorescence detection (Ex/Em=530/590 nm) which is then correlated to give a Sialic Acid

level mol/mol from the sialic acid standard curve. The resulting amount of sialylation is shown in Table 4.

Table 4: Sialic Acid Content of Human Serum IgM

| Antibody | SA (mol/mol) |
|---|---|
| Human serum IgM | 30 |

Example 5: Effect of increased sialylation on IGM antibodies

**[0288]** The glycoengineered IgM CD20 x CD3 IGM-A ("anti-CD20 x CD3 IGM-A-GEM") material used in the experiments in this example had about 37 moles sialic acid/mole of IgM and was prepared as described in Example 1. The non-glycoengineered IGM CD20 x CD3 IGM-A had about 14 moles sialic acid/mole of IgM.

Complement dependent cytotoxicity

**[0289]** The CD20-expressing Ramos (ATCC cat. #CRL-1596) cells were cultured in RPMI (Invitrogen) supplemented with 10% Heat inactivated Fetal Bovine Serum (Gibco Cat. # 16140-071). Ramos cells (50,000) were seeded in a 96-well plate in 10ul/well volume. Cells were treated with 10ul/well serial dilutions of 2 different lots of anti-CD20 x CD3 IGM, or anti-CD20 x CD3 IGM-A sialylated *in vitro* as described in Example 1 ("anti-CD20 x CD3 IGM-A-GEM"). All the antibody dilutions were carried out in RPMI media supplemented with 10% heat inactivated serum. Human serum complement (Quidel cat. #A113) was added to the antibody-treated cells at a final concentration of 5% in 10ul/well volume. The reaction mixtures were incubated at 37 °C for 4 hours. CELLTITER-GLO® reagent (Promega cat. #G7572) was added at a volume equal to the volume of culture medium present in each well. The plate was shaken for 2 minutes, incubated for 10 minutes at room temperature, and luminescence was measured on an Envision multimode reader (Perkin Elmer) using 0.1 s integration time per well. Data were analyzed using GraphPad Prism and a four-parameter fit with top and bottom values fixed at 100 and 0% viability, respectively. The concentration of antibody that yields a half-maximal response ($EC_{50}$) was calculated for each condition and is shown in Table 5. *In vitro* sialylation had no appreciable effect on complement-dependent cytotoxicity.

Table 5: CDC Activity

| Sample | $EC_{50}$ (pM) |
|---|---|
| anti-CD20 x CD3 IGM-A | 260 |
| anti-CD20 x CD3 IGM -A-GEM | 230 |

T-cell Activation

**[0290]** T cell activation (TCA) by anti-CD20 x CD3 IGM-A-GEM or anti-CD20 x CD3 IGM-A was determined using a luminescence-based readout in the presence of antigen-positive Jurkat based reporter cells. Engineered Jurkat T-cells (Promega J1601 Part# J131A) and Ramos cells were cultured in RPMI (Invitrogen) supplemented with 10% Heat inactivated Fetal Bovine Serum (Gibco Cat. # 16140-071). Ramos cells (7500 cells /well in 10ul volume) were added in a white 384 well assay plate. Next, serial dilutions of anti-CD20 x CD3 IGM-A-GEM or anti-CD20 x CD3 IGM-A were added to Ramos cells in 10 µl volume. The engineered Jurkat cells (25000 cells /well in 20 µl volume) were added to mixture to final volume of 40 µL. The mixture was incubated for 16h at 37 ºC with 5% $CO_2$. The cell mixtures were then mixed with 20 µL lysis buffer containing luciferin (Promega, CELLTITER-GLO®) to measure luciferase reporter activity. Light output was measured by EnVision plate reader. $EC_{50}$ was determined by 4 parameter curve fit using Prism software.

**[0291]** The $EC_{50}$ was calculated for each condition and is shown in Table 6. *In vitro* sialylation had no appreciable effect on T cell activation.

Table 6: T cell Activation

| Sample | $EC_{50}$ (pM) |
|---|---|
| anti-CD20 x CD3 IGM-A | 29 |
| anti-CD20 x CD3 IGM-A-GEM | 22 |

B-cell killing *in vitro*

**[0292]** Ramos, a CD19+CD20+ B cell line, was labeled with a cell tracing dye (Oregon Green 488, ThermoFisher, Cat# C34555), and then co-cultured with primary human CD8+ T cells (Precision for Medicine, Cat# 84300; Negatively selected) with serial dilutions of anti-CD20 x CD3 IGM-A or anti-CD20 x CD3 IGM-A-GEM for 48 hours at 37 ºC, 5% $CO_2$. Cells were harvested and stained with 7-AAD (BD Biosciences, Cat# 559925) and analyzed by flow cytometry to assess viable B cells. The $EC_{50}$ was calculated for each

condition and is shown in Table 7. *In vitro* sialylation had no appreciable effect on the ability of the antibody to kill B cells.

Table 7: T-Cell dependent B Cell Killing

| **Sample** | **Max (%)** | **$EC_{50}$ (pM)** |
|---|---|---|
| anti-CD20 x CD3 IGM-A | 94.5 | 6.57 |
| anti-CD20 x CD3 IGM-A-GEM | 94.8 | 7.99 |

Pharmacokinetics

**[0293]** Pharmacokinetic parameters were measured for various IgM antibodies in an *in vivo* mouse model as follows. Balb/c mice were injected with 5 mg/kg of either anti-CD20 x CD3 IGM-A or anti-CD20 x CD3 IGM-A-GEM antibody via intravenous infusion. Blood samples were collected at 10 or 12 time points total for each antibody, with 2 mice per time point. Each mouse was bled once through the facial vein (100 µL) and then another time by terminal cardiac puncture (max obtainable, ~500 µL). A standard ELISA assay was used to measure the serum concentration of each antibody in the blood at each time point. Quality metrics were verified on all ELISAs, and PK parameters, including $T_{1/2\text{-alpha}}$, $T_{1/2\text{-beta}}$, and the area under the concentration curve from time zero to infinity ($AUC_{0\text{-}\infty}$, measured in µg/ml*hr) were derived using standard curve fitting techniques (Win Non Lin, Phoenix Software). The PK results, including area under the curve (AUC) are presented in **FIG. 6**.

Example 6: Cell line engineering to increase sialylation

**[0294]** A vector for comprising a GACACC Kozak sequence, a sequence encoding an alpha-2,6-sialyltransferase (ST6) SEQ ID 3 (NCBI Reference Sequence: sp|P15907.1), and a hygromycin marker selection was generated by standard methods. The vector was electroporated into a stable CHO subclone expressing anti-CD20 x CD3 IGM-A. After selection and recovery, the resulting pool was subcloned by limiting dilution into 384-well plates. Phenotypic screening was done by labelling the subcloned cells with SNA-1 conjugated to fluorescein isothiocyanate (FITC). SNA-1 is a lectin specific to 2,6-sialic acid. The cells themselves were labeled directly after being washed in FACS buffer. The fluorescence levels were detected, and the results are shown in **FIG. 7**. Only 4 of 60

subclones generated a signal above that of HEK293 cells, which were used as a positive control. Two subclones 25 and 47 were picked for further studies.

**[0295]** To detect the presence of ST6 in the genome of the subclones 25 and 47, QPCR analysis was done using the primers in Table 8.

Table 8: Primers used in a QPCR assay.

| | |
|---|---|
| Primer 1 | GAC CGA CGT GTG CTA CTA TTA C (SEQ ID NO: 39) |
| Primer 2 | GAG GTG CTT CAC GAG ATT CTT (SEQ ID NO 40) |

**[0296]** These primers are represented in the coding sequence (CDS) of the ST6 gene. Both subclones generated a positive response by the 38$^{th}$ cycle, whereas a CHO cell control did not.

**[0297]** A western blot was performed to detect 2,6 linked sialic acid in the anti-CD20 x CD3 IGM-A antibodies expressed and purified from small scale fermentations. The reduced, denatured gel (BioRad® CriterionTGX Stain-Free Precast gel) was visualized and imaged according to manufacturer's instructions. The resulting image is shown in **FIG. 8A**. The gel was then treated with a biotinylated SNA-I lectin and a streptavidin horseradish peroxidase fusion protein and was imaged. The resulting image is shown in **FIG.8B**.

**[0298]** The selected subclones had detectable levels of 2,6-sialic acid; whereas the stable pool of CHO cells from which the subclones originated did not.

**[0299]** Subclone 25 was expanded for a 3-liter bioreactor production run so that a comparison study could be made against the parental anti-CD20 x CD3 IGM-A produced in cells which did not have the 2,6-sialyltransferase gene. **FIGS. 9A-D** shows how the cultures performed in terms of viable cell density (**FIG. 9A**), cell-viability (**FIG. 9B**), production of anti-CD20 x CD3 IGM-A (**FIG. 9C**), and the moles of sialic acid per mole of anti-CD20 x CD3 IGM-A (**FIG. 9D**). Subclone 25 produced 25% less anti-CD20 x CD3 IGM-A by day 8 than the parental cell line (**FIG. 9C**), but the sialic acid content was more than doubled and remained elevated (**FIG. 9D**). Day 12 data shows that subclone 25 produced 500 μg/ml (**FIG. 9C**) with a sialic acid content of 37 mol/mol of IgM (**FIG. 9D**).

Example 7: 2,6-Sialic Acid knock-in parental cell line

**[0300]** A vector for stable insertion of alpha-2,6-sialyltransferase (NCBI Reference Sequence: NP_775324.1) and a hygromycin marker selection was generated by a commercial vendor. The vector was electroporated into a CHO suspension cell-line. The resulting stable pool was cloned, and 384 clones were expanded and screened by cytometry. Cell surface-based labeling was done with a 2,6-sialic acid specific lectin (SNA-1) which was chemically conjugated to a Cy5 dye, the results of this assay are shown in **FIG. 10A**. Based on the screen 48 clones which were growing-out well and had high lectin labelling levels were expanded to 24-deep well plates and shaken at 300 RPM in an incubator at 37 °C, 5% $CO_2$, and 80% humidity. An identical 2,6-sialic acid screen was done after the 48 clones had expanded in the deep well plate and the top 22 were transferred to shake flask.

**[0301]** The selected 22 clones were screened again when the cell densities were between 1-4 million cells/ml. Lectin labeling levels in this case are shown in **FIG. 10B** with the corresponding viability of the culture at the time they were assayed.

**[0302]** Based on the shake flask analysis six clones were chosen to be evaluated initially by transfection with two to four control IgMs. The parental CHO cell-line was also transfected with the same four control IgMs and used as a basis for comparison. After transfection four of the six selected clones were either not able to recover or had growth problems after or prior to transfection. Two clones (2B4 and 2C2) which were able to survive after transfection, showed higher titer in all but one case, and, in all cases, had higher sialic acid content than IgM's from the parental CHO cell-line. Table 9 shows the result of 7-day fed batch fermentations comparing the parental cell-line to the two 2,6-sialyltransferase clones. Sialic acid content was determined from the protein purified after the harvest. Four different IgMs were transfected into 2B4 and the parental cell-line and two of these IgMs were also transfected into 2C2.

Table 9: Harvest titer data and sialic acid levels on purified proteins from the harvested fermentation.

| | Clone 2B4 | | | | Clone 2C2 | | | |
|---|---|---|---|---|---|---|---|---|
| IgM | Titer (mg/mL) | Fold Difference in Titer Relative to Parental | Sialic Acid (mol/mol) | Fold Difference in Sialic Acid Relative to Parental | Titer (mg/mL) | Fold Difference in Titer Relative to Parental | Sialic Acid (mol/mol) | Fold Difference in Sialic Acid Relative to Parental |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | 2875 | 1.3 | 51.3 | 1.7 | | | | |
| 2 | 1078 | 1.6 | 46.0 | 1.5 | 379 | 0.6 | 37.2 | 1.2 |
| 3 | 752 | 1.6 | 41.4 | 1.6 | 727 | 1.5 | 40.0 | 1.5 |
| 4 | 1500 | 1.3 | 31.8 | 1.4 | | | | |

**[0303]** To further characterize the clones, 2,6-sialic acid and 2,3-sialic acid levels were measured on the cell surface by cytometry. Measurement was made using fluorescently conjugated lectins which are specific to either form of sialic acid. All viabilities were over 95% at the time the labeling was carried-out. HEK293 cells, the CHO parental cell-line, selected clones, and IgM transfected clones were all analyzed in an identical manner. **FIGS. 11A** and **11B** show the 2,3-sialic acid and 2,6-sialic acid levels for untransfected cells, respectively. **FIG. 11C** compares the 2,3-sialic acid and 2,6-sialic acid levels in untransfected and IgM #4 transfected parental and 2B4 cells. The data shown in **FIG. 11A** indicates that the CHO parental cell had higher 2,3-sialic acid levels then HEK293 cells or the clones transfected with 2,6-sialyltransferase. The data shown in **FIG. 11B** indicates the 2,6-sialic acid levels on the clones was elevated well above the level of the CHO parental. **FIG. 11C** indicates that the IgM transfected cell-lines retained high levels of 2,6-sialic acid.

Example 8: *in vitro* sialylation under various conditions

**[0304]** Varying amounts of truncated human ST6 were added to a reaction solution comprising an IgM antibody and cytidine-5′-monophospho-N-acetylneuraminic acid sodium salt (CMP-NANA, 1.5 mg/ml) in ratios as shown in Table 10. The durations and temperatures for each reaction, and the resulting sialylation (quantitated as described in Example 1) are also shown in Table 10. Room temperature (RT) is 15 to 25 °C.

Table 10: *in vitro* sialylation conditions and results

| Condition # | Antibody in reaction | Temp. (°C) | Time (h) | Antibody: CMP-NANA: ST6 (mass) | Sialic Acid (mol/mol) |
|---|---|---|---|---|---|
| 1 | anti-CD20 x CD3 IGM-A | 37 | 24 | - | 20 |
| 2 | anti-CD20 x CD3 IGM-A | 37 | 24 | 6:3:1 | 62 |
| 3 | Target 1 IGM-D | 37 | 6 | - | 27 |
| 4 | Target 1 IGM-D | 37 | 6 | 500:1000:1 | 47 |
| 5 | Target 2 IGM-E | 37 | 6 | - | 32 |
| 6 | Target 2 IGM-E | 37 | 6 | 100:50:1 | 62 |

| | | | | | |
|---|---|---|---|---|---|
| 7 | anti-CD20 x CD3 IGM-F | 37 | 7 | - | 20 |
| 8 | anti-CD20 x CD3 IGM-F | 37 | 7 | 100:50:1 | 54 |
| 9 | anti-CD20 x CD3 IGM-F | 37 | 7 | 100:50:1 | 64 |
| 10 | anti-CD20 x CD3 IGM-A | RT | 21 | 100:50:1 | 53 |
| 11 | anti-CD20 x CD3 IGM-A | RT | 21 | 100:25:1 | 51 |
| 12 | anti-CD20 x CD3 IGM-A | RT | 21 | 100:10:1 | 51 |
| 13 | anti-CD20 x CD3 IGM-A | RT | 21 | 1000:500:1 | 42 |
| 14 | anti-CD20 x CD3 IGM-A | RT | 21 | 1000:250:1 | 41 |
| 15 | anti-CD20 x CD3 IGM-A | RT | 21 | 1000:100:1 | 44 |
| 16 | anti-CD20 x CD3 IGM-A | RT | 20 | 100:50:1 | 48 |
| 17 | anti-CD20 x CD3 IGM-A | RT | 20 | 500:62.5:1 | 51 |
| 18 | anti-CD20 x CD3 IGM-A | RT | 20 | 1000:500:1 | 42 |
| 19 | anti-CD20 x CD3 IGM-A | RT | 20 | - | 16 |
| 20 | anti-CD20 x CD3 IGM-A | RT | 21 | 10000:5000:1 | 34 |
| 21 | anti-CD20 x CD3 IGM-A | RT | 21 | 3000:1500:1 | 39 |
| 22 | anti-CD20 x CD3 IGM-A | RT | 21 | 1000:500:1 | 42 |
| 23 | anti-CD20 x CD3 IGM-A | RT | 21 | 100:50:1 | 48 |
| 24 | anti-CD20 x CD3 IGM-A | 37 | 20 | - | 15 |
| 25 | anti-CD20 x CD3 IGM-A | 37 | 7 | 100:50:1 | 54 |
| 26 | anti-CD20 x CD3 IGM-A | RT | 20 | 100:50:1 | 59 |
| 27 | anti-CD20 x CD3 IGM-A | RT | 22 | 1000:50:1 | 52 |
| 28 | anti-CD20 x CD3 IGM-A | 4 | 8 | 100:50:1 | 53 |
| 29 | anti-CD20 x CD3 IGM-A | 15 | 48 | 100:50:1 | 54 |
| 30 | anti-CD20 x CD3 IGM-A | 37 | 24 | 100:50:1 | 50 |
| 31 | anti-CD20 x CD3 IGM-A | 37 | 24 | 5000:2500:1 | 45 |
| 32 | anti-CD20 x CD3 IGM-A | RT | 48 | 100:50:1 | 54 |
| 33 | anti-CD20 x CD3 IGM-A | RT | 48 | 250:125:1 | 50 |
| 34 | anti-CD20 x CD3 IGM-A | RT | 48 | 1000:500:1 | 45 |
| 35 | anti-CD20 x CD3 IGM-A | RT | 48 | 2500:1250:1 | 44 |
| 36 | anti-CD20 x CD3 IGM-A | RT | 48 | 5000:2500:1 | 40 |

**[0305]** The antibodies from Conditions 1 and 2 were compared by size exclusion chromatography (SEC), dynamic light scattering (DLS), hybrid gel, reducing gel and the CDC and TCA assays described in Example 3, and the *in vitro* sialyation did not change the SEC profile, the dynamic radius, or the mobility of the antibody, and the antibodies had similar activity TCA and CDC activity (data not shown).

**[0306]** The antibodies from Conditions 19-23 were also compared by the TCA assay described in Example 3, and the data are shown in **FIG. 12**. All antibodies assayed had similar TCA activity.

**[0307]** The SA levels of the antibodies from Conditions 29-31 were monitored at set timepoints throughout the reaction. The SA levels over 48 hours or over the first 15 hours are plotted in **FIGS. 13A** and **13B**, respectively. For the antibody:CMP-NANA:ST-6 mass

ratios of 100:50:1, maximum sialyation was reached with 1 hour and there was little difference observed between 37 °C and 15 °C.

**[0308]** The SA levels of the antibodies from Conditions 32-36 were monitored at set timepoints throughout the reaction. The SA levels over 48 hours are plotted in **FIG. 14**. Antibody:CMP-NANA:ST-6 mass ratios of 100:50:1 achieved sialyation of >60 mol/mol SA by 18 h at RT and did not drop significantly for up to 48 hours at RT. Antibody:CMP-NANA:ST-6 mass ratios of 250:125:1 resulted in a slower rise in SA levels and reached >60 mol/mol SA after 36 h. For all ratios, no significant desialylation was observed at RT up to 36 hrs. At antibody:CMP-NANA:ST-6 mass ratios of 5000:2500:1, 40 mol/mol SA was achieved, and the antibody population was not sialylated to the maximum extent possible.

Example 9: Pharmacokinetics of IgM antibodies with high sialic acid levels

**[0309]** Pharmacokinetic parameters were measured for various IgM antibodies in an *in vivo* mouse model as follows. Balb/c mice were injected with 5 mg/kg of anti-CD20 x CD3 IGM-A, anti-CD20 x CD3 IGM-A-GEM antibodies of various sialic acid levels, anti-CD20 x CD3 IGM-F, anti-CD20 x CD3 IGM-F-GEM antibodies of various sialic acid levels, or human serum IgM via intravenous infusion. Blood samples were collected at 10 or 12 time points total for each antibody, with at least 2 mice per time point. Each mouse was bled once through the facial vein (100 μL) and then another time by terminal cardiac puncture (max obtainable, ~500 μL). A standard ELISA assay was used to measure the serum concentration of each antibody in the blood at each time point. Quality metrics were verified on all ELISAs, and PK parameters, including $T_{1/2\text{-alpha}}$, $T_{1/2\text{-beta}}$, and the area under the concentration curve from time zero to infinity ($AUC_{0\text{-}\infty}$, measured in μg/ml*hr) were derived using standard curve fitting techniques (Win Non Lin, Phoenix Software). The sialic acid levels and the resulting $AUC_{0\text{-}\infty}$ for each antibody are shown in **FIG. 15**.

Example 10: IgM antibodies with high sialic acid levels activity in cynomolgus monkeys

**[0310]** Pharmacokinetic parameters and cellular markers were measured for IgM antibodies in an *in vivo* cynomolgus monkey model as follows. Cynomolgus primates were injected with 10 mg/kg of anti-CD20 x CD3 IGM-F (SA 18 mol/mol) (2 animals), anti-CD20 x CD3 IGM-F (SA 9 mol/mol) (2 animals), or anti-CD20 x CD3 IGM-F-GEM (SA 51

mol/mol) (4 animals) antibodies. Blood samples were collected at 12 time points total for each antibody. A standard ELISA assay was used to measure the serum concentration of each antibody in the blood at each time point. Quality metrics were verified on all ELISAs, and PK parameters, including $T_{1/2\text{-alpha}}$, $T_{1/2\text{-beta}}$, and the area under the concentration curve from time zero to infinity ($AUC_{0-\infty}$, measured in μg/ml*hr) were derived using standard curve fitting techniques (Win Non Lin, Phoenix Software). Flow cytometry was used to measure cellular markers. The PK results for anti-CD20 x CD3 IGM-F (SA 18 mol/mol) and 2 of the 4 anti-CD20 x CD3 IGM-F-GEM (SA 51 mol/mol) treated animals are presented in **FIG. 16**. The $AUC_{0-\infty}$ for the high sialic acid antibody was twofold higher than for the low sialic acid antibody. The relative numbers of B cells at each time point are shown in **FIG. 17A**, and the day that B cells began to recover is shown in **FIG. 17B**.

Example 11: *in vitro* sialylation with multiple enzymes

**[0311]** The combination of galactosylation and sialylation was compared to sialylation only.

**[0312]** Sialylation only was completed by mixing 120 μg anti-CD20 x CD3 IGM-A antibody with 1 mol/mol SA or 21 mol/mol SA, 60 μg of CMP NANA, and 20 μg ST6 (a mass ratio of IgM:CMP NANA:ST6 of 6:3:1). The sample was then incubated for 24 hours at 37 °C.

**[0313]** The combination of galactosylation and sialylation was completed by mixing 1 μg β-1,4-Galactosyltransferase, 60 μg UDP-Galactose, 60 μg anti-CD20 x CD3 IGM-A antibody with 1 mol/mol SA or 21 mol/mol SA (a mass ratio of IgM: UDP-Galactose: β-1,4-Galactosyltransferase of 60:60:1). The mixture was incubated for 7 hours at 37 °C. 30 μg CMP NANA and 1 μg of ST6 were then added to the mixture and was incubated for 20 hours at 37 °C.

**[0314]** The resulting sialic acid levels are shown in Table 11

Table 11: Sialic levels after sialylation with or without galactosylation

| Starting Material | Treatment | Sialic Acid (mol/mol) |
|---|---|---|
| anti-CD20 x CD3 IGM-A 1 mol/mol SA | Sialylation only | 44 |
| anti-CD20 x CD3 IGM-A 1 mol/mol SA | Galactosylation and Sialylation | 65 |
| anti-CD20 x CD3 IGM-A 21 mol/mol SA | Sialylation only | 60 |

| | | |
|---|---|---|
| anti-CD20 x CD3 IGM-A 21 mol/mol SA | Galactosylation and Sialylation | 65 |

**[0315]** The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary embodiments or examples but should be defined only in accordance with the following claims and their equivalents.

Table 12: Sequences in the Disclosure

| SEQ ID | Nickname (source) | Sequence |
|---|---|---|
| 1 | Human IgM Constant region IMGT allele IGHM*03 (GenBank: pir\|S37768\|) | GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPS VLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVF VPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPT TYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFA SIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASI CEDDWNSGERFTCTVTHTDLPSPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESAT ITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWN TGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY |
| 2 | Human IgM Constant region IMGT allele IGHM*04 (GenBank: sp\|P01871.4\|) | GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPS VLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVF VPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPT TYKVTSTLTIKESDWLGQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFA SIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASI CEDDWNSGERFTCTVTHTDLPSPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESAT ITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWN TGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY |
| 3 | Human ST6Gal I, Sialyltransferase 1 (GenBank: sp\|P15907.1\|) | MIHTNLKKKFSCCVLVFLLFAVICVWKEKKKGSYYDSFKLQTKEFQVLKSLGKLAMGS DSQSVSSSSTQDPHRGRQTLGSLRGLAKAKPEASFQVWNKDSSSKNLIPRLQKIWKNY LSMNKYKVSYKGPGPGIKFSAEALRCHLRDHVNVSMVEVTDFPFNTSEWEGYLPKESI RTKAGPWGRCAVVSSAGSLKSSQLGREIDDHDAVLRFNGAPTANFQQDVGTKTTIRLM NSQLVTTEKRFLKDSLYNEGILIVWDPSVYHSDIPKWYQNPDYNFFNNYKTYRKLHPN QPFYILKPQMPWELWDILQEISPEEIQPNPPSSGMLGIIIMMTLCDQVDIYEFLPSKR KTDVCYYYQKFFDSACTMGAYHPLLYEKNLVKHLNQGTDEDIYLLGKATLPGFRTIHC |
| 4 | Human B4GALT4, HUMAN Beta-1,4-galactosyltransferase (GenBank: sp\|O60513\|) | MGFNLTFHLSYKFRLLLLLTLCLTVVGWATSNYFVGAIQEIPKAKEFMANFHKTLILG KGKTLTNEASTKKVELDNCPSVSPYLRGQSKLIFKPDLTLEEVQAENPKVSRGRYRPQ ECKALQRVAILVPHRNREKHLMYLLEHLHPFLQRQQLDYGIYVIHQAEGKKFNRAKLL NVGYLEALKEENWDCFIFHDVDLVPENDFNLYKCEEHPKHLVVGRNSTGYRLRYSGYF GGVTALSREQFFKVNGFSNNYWGWGGEDDDLRLRVELQRMKISRPLPEVGKYTMVFHT RDKGNEVNAERMKLLHQVSRVWRTDGLSSCSYKLVSVEHNPLYINITVDFWFGA |
| 5 | Precursor Human J Chain | MKNHLLFWGVLAVFIKAVHVKAQEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVER NIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDE DSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD |
| 6 | Mature Human J Chain | QEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLR TRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPL VYGGETKMVETALTPDACYPD |
| 7 | J Chain Y102A mutation | QEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLR TRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCATYDRNKCYTAVVPL VYGGETKMVETALTPDACYPD |
| 8 | anti-CD20 x CD3 IGM-A VH | EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDT RYSPSFQGQVTISADKSITTAYLQWSSLKASDTAMYYCARHPSYGSGSPNFDYWGQGT LVTVSS |

| SEQ ID | Nickname (source) | Sequence |
|---|---|---|
| 9 | anti-CD20 x CD3 IGM-A VL | DIVMTQTPLSSPVTLGQPASISCRSSQSLVYSDGNTYLSWLQQRPGQPPRLLIYKISN RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCVQATQFPLTFGGGTKVEIK |
| 10 | anti-DR5 IgM-B VH | EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYVMSWVRQAPGKGLEWVATISSGGSYT YYPDSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCARRGDSMITTDYWGQGTLVT VSSA |
| 11 | anti-DR5 IgM-B VL | DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYWASTRHTGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSSYRTFGQGTKVEIK |
| 12 | anti-DR5 IgM-C VH | QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYFWSWIRQLPGKGLECIGHIHNSGT TYYNPSLKSRVTISVDTSKKQFSLRLSSVTAADTAVYYCARDRGGDYYYGMDVWGQGT TVTVSS |
| 13 | anti-DR5 IgM-C VL | EIVLTQSPGTLSLSPGERATLSCRASQGISRSYLAWYQQKPGQAPSLLIYGASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQFGSSPWTFGQGTKVEIK |
| 14 | SP34 VH | EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNY ATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWG QGTLVTVSS |
| 15 | SP34 VH CDR1 | GFTFNTYAMN |
| 16 | SP34 VH CDR2 | ARIRSKYNNYATYYADSVKD |
| 17 | SP34 VH CDR3 | VRHGNFGNSYVSWFAY |
| 18 | SP34 VL | QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNKRAP GVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVL |
| 19 | SP34 VL CDR1 | RSSTGAVTTSNYAN |
| 20 | SP34 VL CDR2 | GTNKRAP |
| 21 | SP34 VL CDR3 | ALWYSNL |
| 22 | A-55-VH (WO2018208864) | EVQLLESGGGLVQPGGSLRLSCAASGFTFDTYAMNWVRQAPGKGLEWVARIRSKYNNY ATYYADSVKDRFTISRDDSKSTLYLQMESLRAEDTAVYYCVRHGNFQGGYVSWFAHWG QGTLVTVSS |
| 23 | A-55-VL (WO2018208864) | QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAP GVPDRFSGSLLGDKAALTITGAQAEDEADYYCALWYSNHWVFGGGTKLTVL |
| 24 | A-55-scFv | EVQLLESGGGLVQPGGSLRLSCAASGFTFDTYAMNWVRQAPGKGLEWVARIRSKYNNY ATYYADSVKDRFTISRDDSKSTLYLQMESLRAEDTAVYYCVRHGNFQGGYVSWFAHWG QGTLVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNY ANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQAEDEADYYCAL WYSNHWVFGGGTKLTVL |
| 25 | A-56-VH (WO2018208864) | EVQLLESGGGLVQPGGSLRLSCAASGFTFDTYAMNWVRQAPGKGLEWVARIRSKYNNY ATYYADSVKDRFTISRDDSKSTLYLQMESLRAEDTAVYYCVRHGNFGGGYVSWFAWWG QGTLVTVSS |
| 26 | A-56-V (WO2018208864) | QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAP GVPDRFSGSLLGDKAALTITGAQAEDEADYYCALWYSNHWVFGGGTKLTVL |
| 27 | A-56-scFv | EVQLLESGGGLVQPGGSLRLSCAASGFTFDTYAMNWVRQAPGKGLEWVARIRSKYNNY ATYYADSVKDRFTISRDDSKSTLYLQMESLRAEDTAVYYCVRHGNFGGGYVSWFAWWG QGTLVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNY ANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQAEDEADYYCAL WYSNHWVFGGGTKLTVL |
| 28 | A-57-VH (WO2018208864) | EVQLLESGGGLVQPGGSLRLSCAASGFTFDTYAMNWVRQAPGKGLEWVARIRSKYNNY ATYYADSVKDRFTISRDDSKSTLYLQMESLRAEDTAVYYCVRHANFGAGYVSWFAHWG QGTLVTVSS |
| 29 | A-57-VL (WO2018208864) | QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAP GVPDRFSGSLLGDKAALTITGAQAEDEADYYCALWYSNHWVFGGGTKLTVL |
| 30 | A-57-scFv | >Adi26957_scFv EVQLLESGGGLVQPGGSLRLSCAASGFTFDTYAMNWVRQAPGKGLEWVARIRSKYNNY ATYYADSVKDRFTISRDDSKSTLYLQMESLRAEDTAVYYCVRHANFGAGYVSWFAHWG QGTLVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNY ANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQAEDEADYYCAL WYSNHWVFGGGTKLTVL |

| SEQ ID | Nickname (source) | Sequence |
|---|---|---|
| 31 | A-55-J* | >X26955J(Y102A)<br>EVQLLESGGGLVQPGGSLRLSCAASGFTFDTYAMNWVRQAPGKGLEWVARIRSKYNNY<br>ATYYADSVKDRFTISRDDSKSTLYLQMESLRAEDTAVYYCVRHGNFQGGYVSWFAHWG<br>QGTLVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNY<br>ANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQAEDEADYYCAL<br>WYSNHWVFGGGTKLTVLGGGGSGGGGSGGGGSQEDERIVLVDNKCKCARITSRIIRSS<br>EDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIV<br>TATQSNICDEDSATETCATYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD |
| 32 | A-56-J* | >X26956J(Y102A)<br>EVQLLESGGGLVQPGGSLRLSCAASGFTFDTYAMNWVRQAPGKGLEWVARIRSKYNNY<br>ATYYADSVKDRFTISRDDSKSTLYLQMESLRAEDTAVYYCVRHGNFGGGYVSWFAWWG<br>QGTLVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNY<br>ANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQAEDEADYYCAL<br>WYSNHWVFGGGTKLTVLGGGGSGGGGSGGGGSQEDERIVLVDNKCKCARITSRIIRSS<br>EDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIV<br>TATQSNICDEDSATETCATYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD* |
| 33 | A-57-J* | >X26957J(Y102A)<br>EVQLLESGGGLVQPGGSLRLSCAASGFTFDTYAMNWVRQAPGKGLEWVARIRSKYNNY<br>ATYYADSVKDRFTISRDDSKSTLYLQMESLRAEDTAVYYCVRHANFGAGYVSWFAHWG<br>QGTLVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNY<br>ANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQAEDEADYYCAL<br>WYSNHWVFGGGTKLTVLGGGGSGGGGSGGGGSQEDERIVLVDNKCKCARITSRIIRSS<br>EDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIV<br>TATQSNICDEDSATETCATYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD* |
| 34 | VJH (WT J) | MGWSYIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQ<br>APGQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCA<br>RSAYYDYDGFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVT<br>ITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQ<br>PEDFATYYCQQWSSNPPTFGGGTKVEIKGGGGSGGGGSGGGGSQEDERIVLVDNKCKC<br>ARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCD<br>PTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTP<br>DACYPDGGGGSGGGGSGGGGSDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFE<br>DHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQE<br>PERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPE<br>LLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAF<br>KAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQD<br>SISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFL<br>GMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQ<br>NLIKQNCELFKQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEA<br>KRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE<br>FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCC<br>KADDKETCFAEEGKKLVAASQAALGL |
| 35 | VJ*H (WT J) | MGWSYIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQ<br>APGQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCA<br>RSAYYDYDGFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVT<br>ITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQ<br>PEDFATYYCQQWSSNPPTFGGGTKVEIKGGGGSGGGGSGGGGSQEDERIVLVDNKCKC<br>ARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCD<br>PTEVELDNQIVTATQSNICDEDSATETCATYDRNKCYTAVVPLVYGGETKMVETALTP<br>DACYPDGGGGSGGGGSGGGGSDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFE<br>DHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQE<br>PERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPE<br>LLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAF<br>KAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQD<br>SISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFL<br>GMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQ<br>NLIKQNCELFKQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEA<br>KRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE |

| SEQ ID | Nickname (source) | Sequence |
|---|---|---|
| | | FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCC KADDKETCFAEEGKKLVAASQAALGL |
| 36 | mature V15J ("WT", or wild-type) | QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGYINPRSGYT HYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCARSAYYDYDGFAYWGQGTLV TVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQKP GKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTF GGGTKLEIKGGGGSGGGGSGGGGSQEDERIVLVDNKCKCARITSRIIRSSEDPNEDIV ERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNIC DEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD |
| 37 | mature V15J* | QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGYINPRSGYT HYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCARSAYYDYDGFAYWGQGTLV TVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQKP GKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTF GGGTKLEIKGGGGSGGGGSGGGGSQEDERIVLVDNKCKCARITSRIIRSSEDPNEDIV ERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNIC DEDSATETCATYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD |
| 38 | SJ* | >SJ* (S=anti CD3 scfv SP34) MGWSYIILFLVATATGVHSEVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQ APGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYY CVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSQAVVTQESALTTSPG ETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKA ALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVLGGGGSGGGGSGGGGSQEDERIV LVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHL SDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCATYDRNKCYTAVVPLVYGGETK MVETALTPDACYPD |
| 39 | ST6 QPCR primer 1 | GAC CGA CGT GTG CTA CTA TTA C |
| 40 | ST6 QPCR Primer 2 | GAG GTG CTT CAC GAG ATT CTT |
| 41 | "5" Peptide linker | GGGGS |
| 42 | "10" Peptide linker | GGGGSGGGGS |
| 43 | "15" Peptide linker | GGGGSGGGGSGGGGS |
| 44 | "20" Peptide linker | GGGGSGGGGSGGGGSGGGGS |
| 45 | "25" Peptide Linker | GGGGSGGGGSGGGGSGGGGSGGGGS |
| 46 | mouse IgM heavy chain constant region (CAC20701.1 ) | ASQSFPNVFPLVSCESPLSDKNLVAMGCLARDFLPSTISFTWNYQNNTEVIQGIRTFP TLRTGGKYLATSQVLLSPKSILEGSDEYLVCKIHYGGKNRDLHVPIPAVAEMNPNVNV FVPPRDGFSGPAPRKSKLICEATNFTPKPITVSWLKDGKLVESGFTTDPVTIENKGST PQTYKVISTLTISEIDWLNLNVYTCRVDHRGLTFLKNVSSTCAASPSTDILNFTIPPS FADIFLSKSANLTCLVSNLATYETLSISWASQSGEPLETKIKIMESHPNGTFSAKGVA SVCVEDWNNRKEFVCTVTHRDLPSPQKKFISKPNEVHKHPPAVYLLPPAREQLNLRES ATVTCLVKGFSPADISVQWKQRGQLLPQEKYVTSAPMPEPGAPGFYFTHSILTVTEEE WNSGETYTCVVGHEALPHLVTERTVDKSTGKPTLYNVSLIMSDTGGTCY |
| 47 | Cynomolgus Monkey presumed IgM constant region sequence (EHH62210.1 ) | ESAGPFKWEPSVSSPNAPLDTNEVAVGCLAQDFLPDSITFSWKFKNNSDISKGVWGFP SVLRGGKYAATSQVLLASKDVMQGTDEHVVCKVQHPNGNKEQNVPLPVVAERPPNVSV FVPPRDGFVGNPRESKLICQATGFSPRQIEVSWLRDGKQVGSGITTDRVEAEAKESGP TTFKVTSTLTVSERDWLSQSVFTCRVDHRGLTFQKNVSSVCGPNPDTAIRVFAIPPSF ASIFLTKSTKLTCLVTDLATYDSVTITWTRQNGEALKTHTNISESHPNGTFSAVGEAS ICEDDWNSGERFRCTVTHTDLPSPLKQTISRPKGVAMHRPDVYLLPPAREQLNLRESA TITCLVTGFSPADIFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEDW NTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVILWTTLSTFVALFVLTLLY SGIVTFIKVR |

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-$X_1$-S/T, wherein N is asparagine, $X_1$ 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-$X_1$-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).

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-$X_1$-S/T, wherein N is asparagine, $X_1$ 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-$X_1$-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.

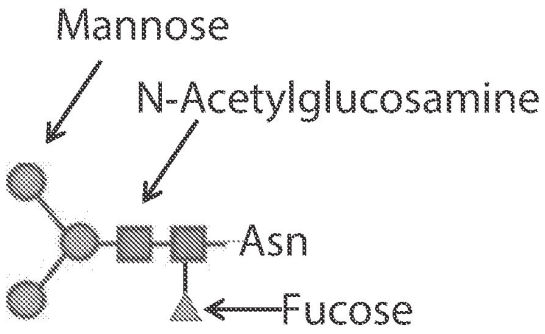
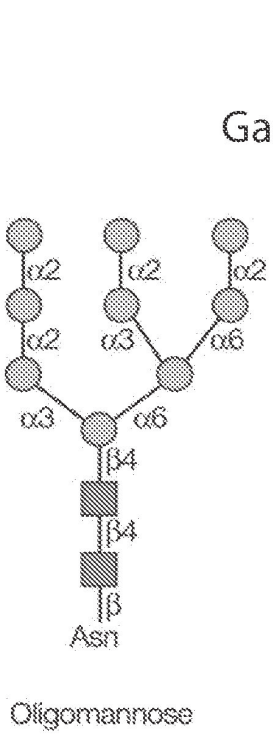
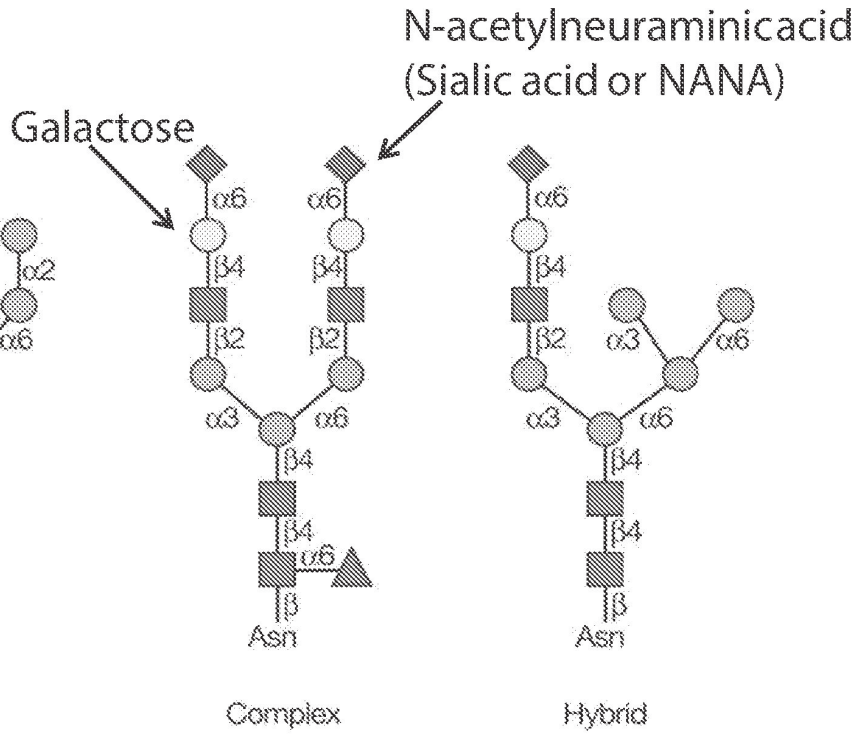



FIG. 1A




Oligomannose

FIG. 1B




Complex

FIG. 1C




Hybrid

FIG. 1D

FIG. 2A

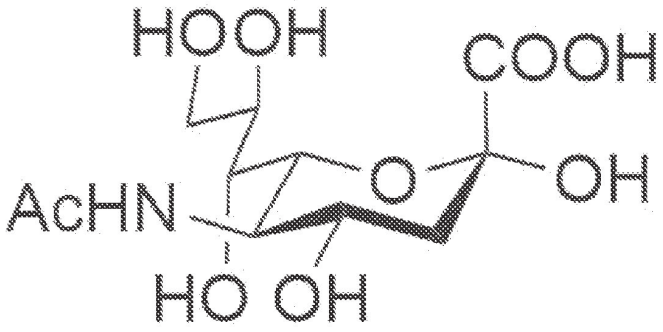

HOOH
COOH
AcHN
O
OH
HO OH

FIG. 2B

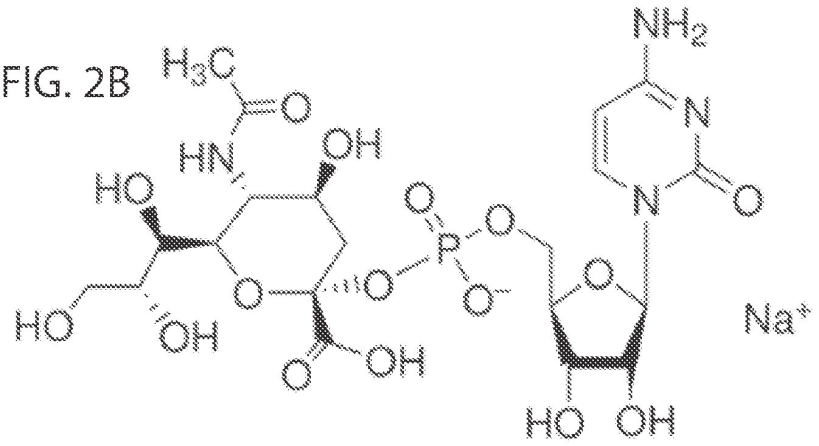

H3C
O
HN
OH
HO
O
P
O
O
O
O
N
O
NH2
N
HO
OH
O
OH
HO
OH
Na+

FIG. 3A

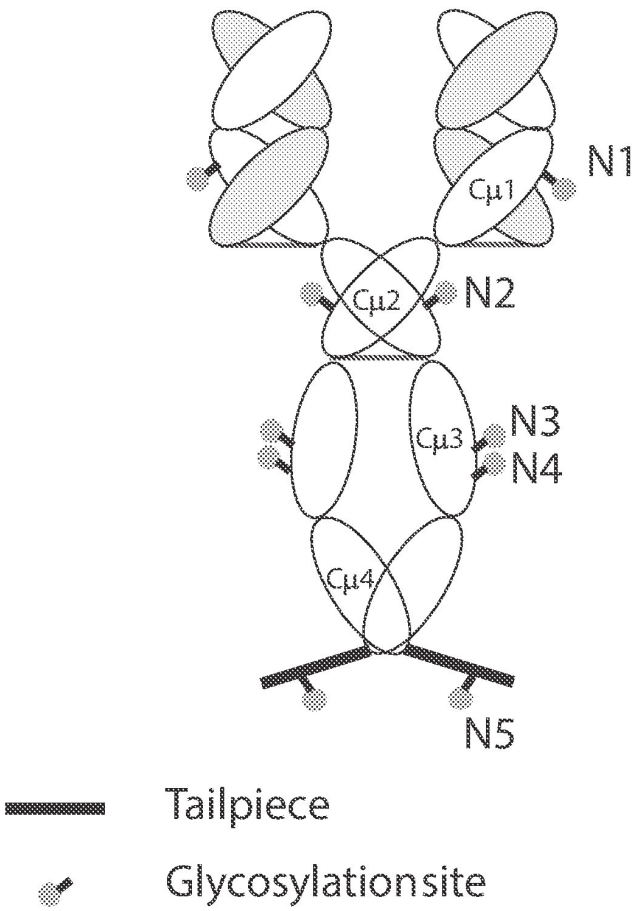


FIGURE 3B


N1
Human GSASAPTLFPPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISS-TRGFPSV 59
Mouse ASQSFPNVFPLVSCESPLSDKNLVAMGCLARDFLPSTISFTWNYQNNTEVIQGIRTFPTL 60
Cynomolgus ESAGPFKWEPSVSSPNAPLDTNEVAVGCLAQDFLPDSITFSWKFKNNSDISKGVWGFPSV 60
Human LRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPP 119
Mouse RTGGKYLATSQVLLSPKSILEGSDEYLVCKIHYGGKNRDLHVPIPAVAEMNPNVNVFVPP 120
Cynomolgus LRGGKYAATSQVLLASKDVMQGTDEHVVCKVQHPNGNKEQNVPLPVVAERPPNVSVFVPP 120
Human RDGFFGN-PRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKV 178
Mouse RDGFSGPAPRKSKLICEATNFTPKPITVSWLKDGKLVESGFTTDPVTIENKGSTPQTYKV 180
Cynomolgus RDGFVGN-PRESKLICQATGFSPRQIEVSWLRDGKQVGSGITTDRVEAEAKESGPTTFKV 179
N2
Human TSTLTIKESDWLGQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLT 237
Mouse ISTLTISEIDWLNLNVYTCRVDHRGLTFLKNVSSTCAASPSTDILNFTIPPSFADIFLS 239
Cynomolgus TSTLTVSERDWLSQSVFTCRVDHRGLTFQKNVSSVCGPNPDTAIRVFAIPPSFASIFLT 238
N3
N4
Human KSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNS 297
Mouse KSANLTCLVSNLATYETLSISWASQSGEPLETKIKIMESHPNGTFSAKGVASVCVEDWNN 299
Cynomolgus KSTKLTCLVTDLATYDSVTITWTRQNGEALKTHTNISESHPNGTFSAVGEASICEDDWNS 298
Human GERFTCTVTHTDLPSPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFS 357
Mouse RKEFVCTVTHRDLPSPQKKFISKPNEVHKHPPAVYLLPPAREQLNLRESATVTCLVKGFS 359
Cynomolgus GERFRCTVTHTDLPSPLKQTISRPKGVAMHRPDVYLLPPAREQLNLRESATITCLVTGFS 358
Human PADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAH 416
Mouse PADISVQWKQRGQLLPQEKYVTSAPMPEPGAPGFYFTHSILTVTEEEWNSGETYTCVVGH 419
Cynomolgus PADIFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEDWNTGETYTCVVAH 418
N5
Human EALPNRVTERTVDKSTGKPTLYNVSLV----------MSDTAGTCY---------- 452
Mouse EALPHLVTERTVDKSTGKPTLYNVSLI----------MSDTGGTCY---------- 455
Cynomolgus EALPNRVTERTVDKSTGKPTLYNVSLVILWTTLSTFVALFVLTLLYSGIVTFIKVR------ 474

FIG. 4

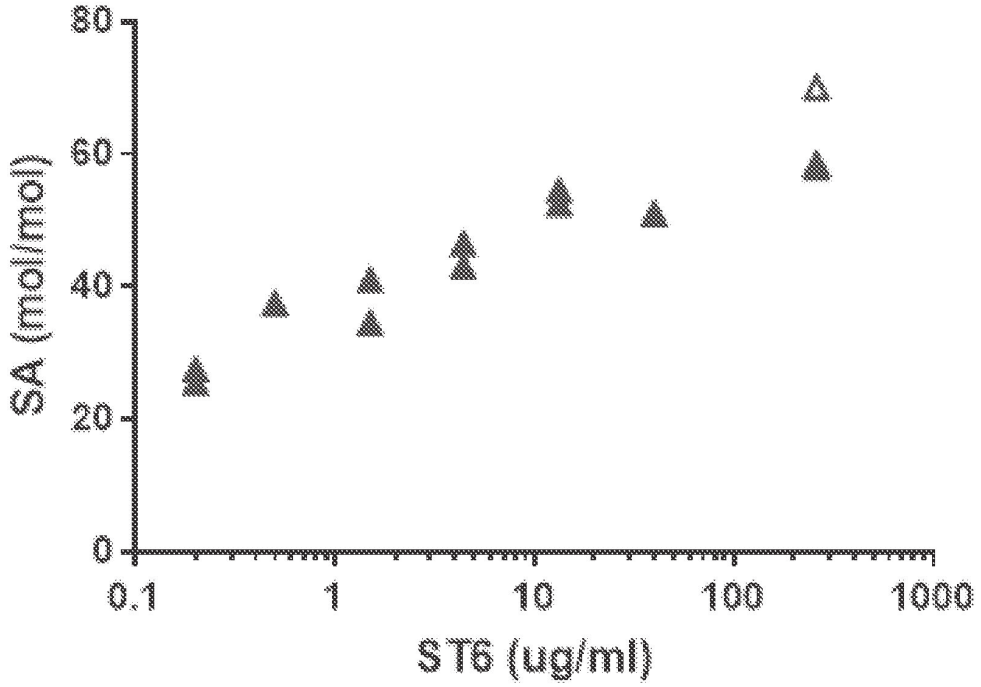


FIG. 5

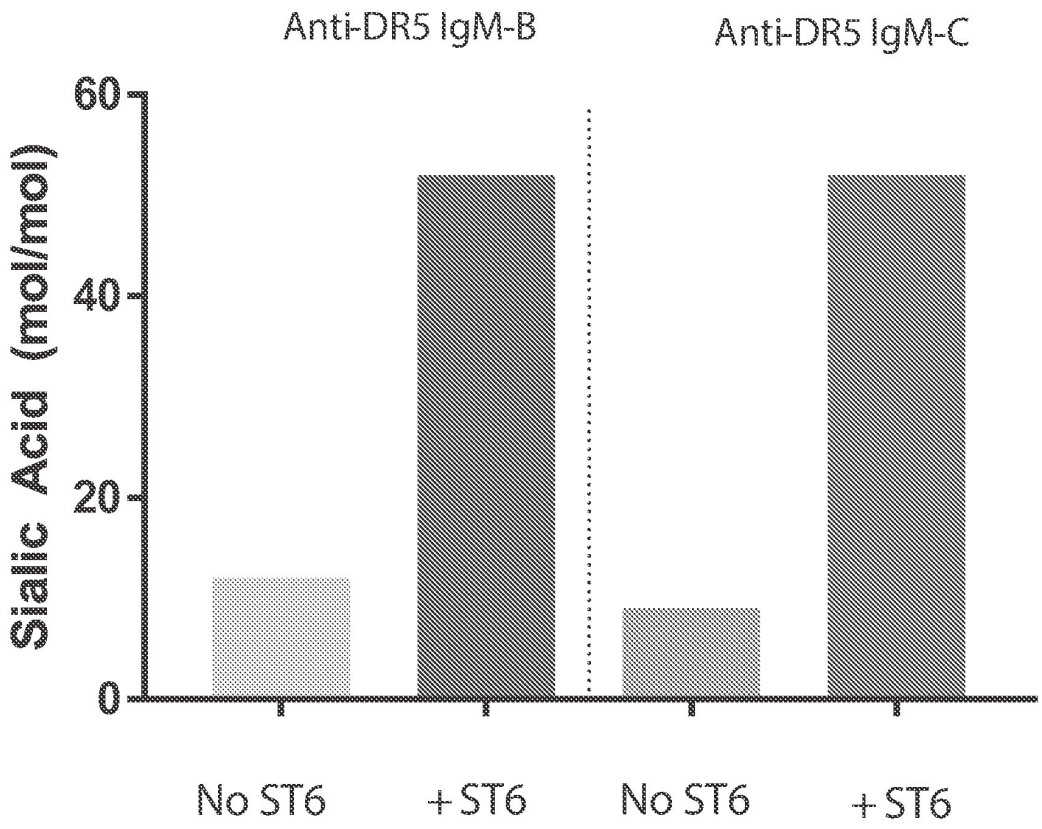

Anti-DR5 IgM-B
Anti-DR5 IgM-C
Sialic Acid (mol/mol)
60
40
20
0
No ST6
+ST6
No ST6
+ST6

FIG. 6

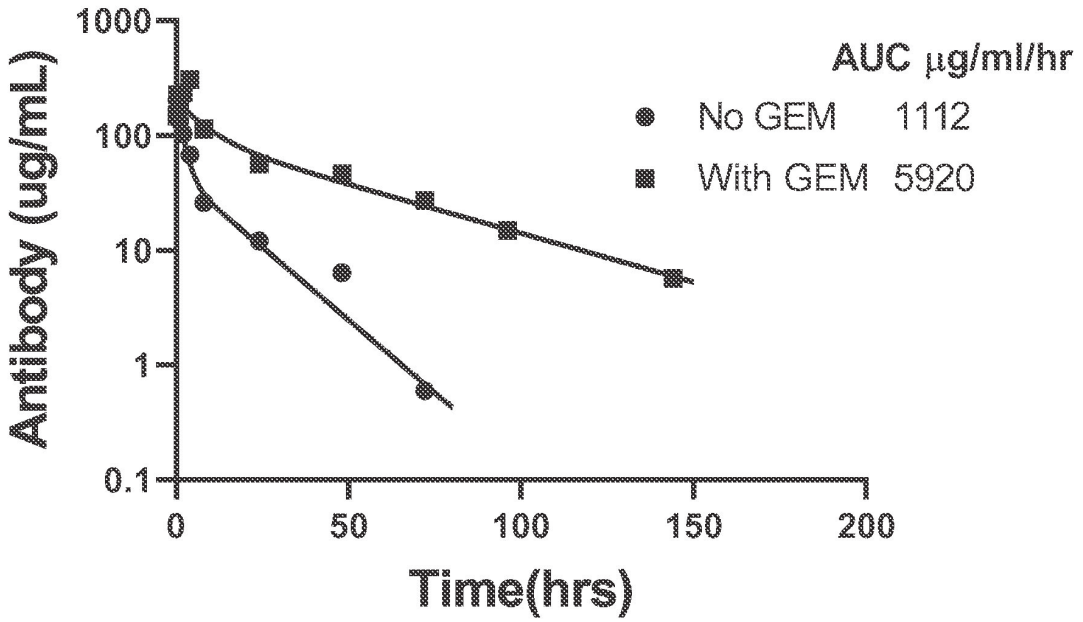

AUC µg/ml/hr
No GEM 1112
With GEM 5920
Antibody (ug/mL)
1000
100
10
1
0.1
0
50
100
150
200
Time(hrs)

FIG. 7

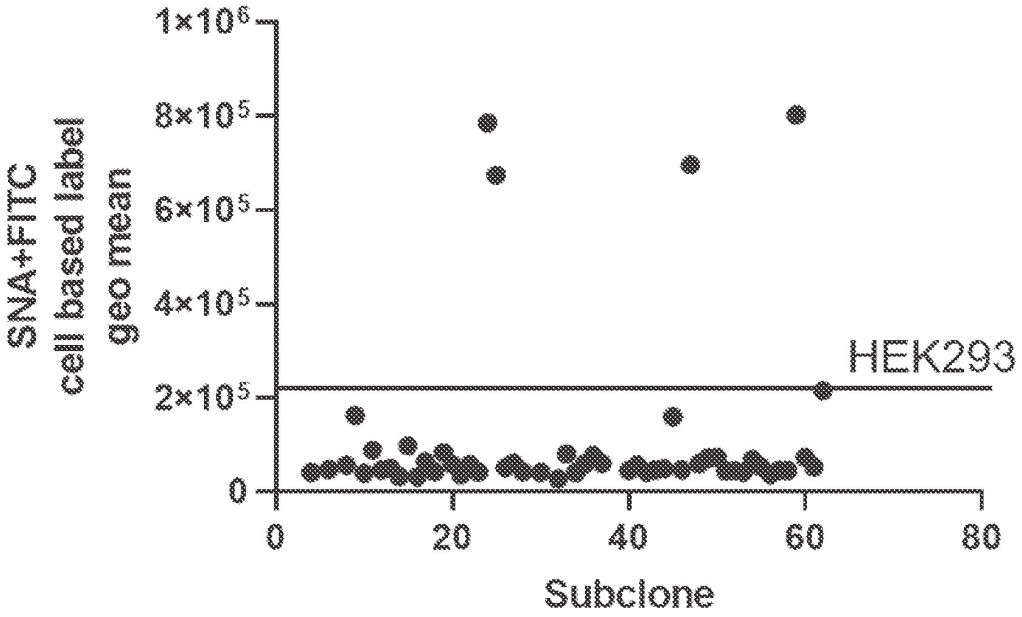

SNA+FITC
cell based label
geo mean
1×10^6
8×10^5
6×10^5
4×10^5
2×10^5
0
HEK293
0
20
40
60
80
Subclone

FIG. 8A

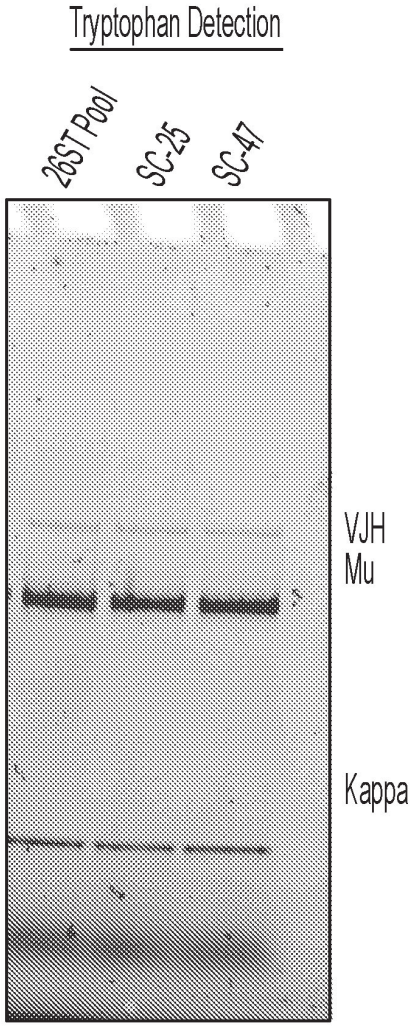

Tryptophan Detection
26ST Pool
SC-25
SC-47
VJH
Mu
Kappa

FIG. 8B

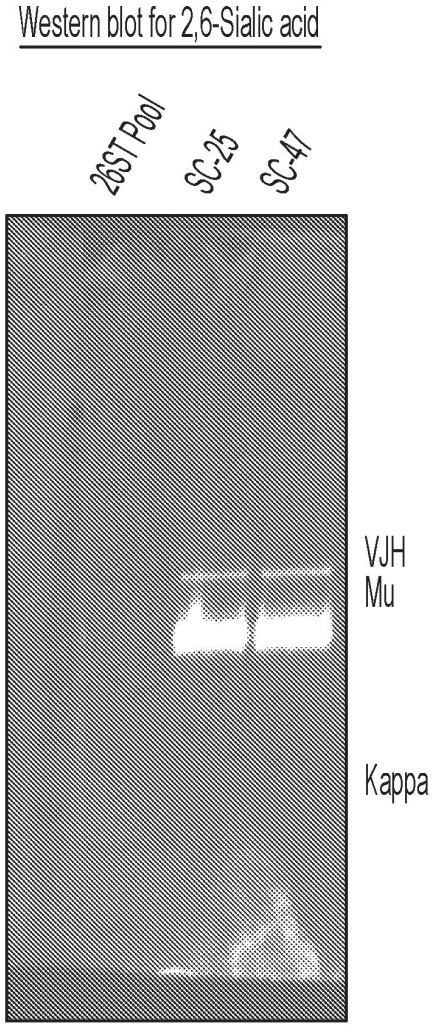

Western blot for 2,6-Sialic acid
26ST Pool
SC-25
SC-47
VJH
Mu
Kappa

FIG. 9A

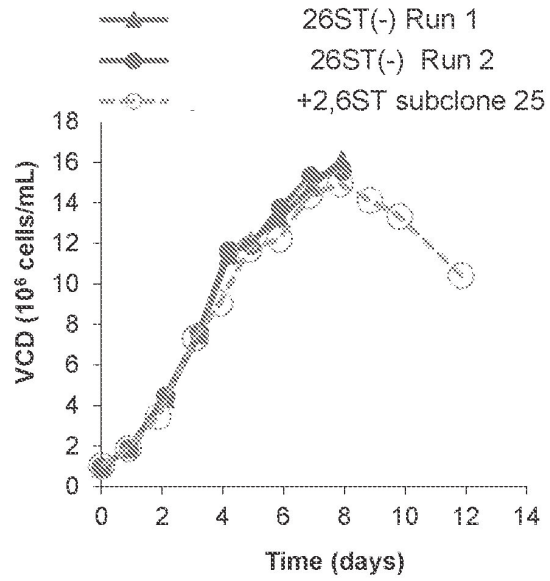



FIG. 9B

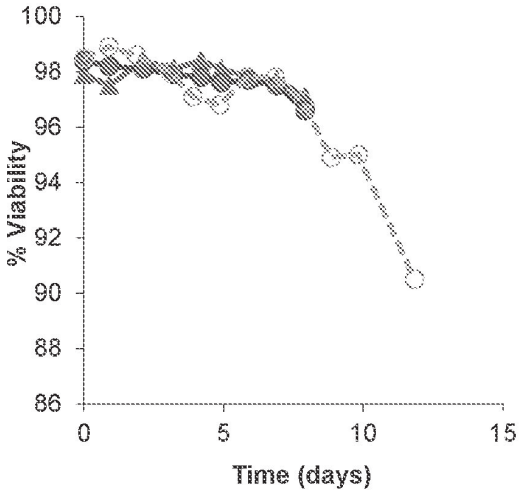



FIG. 9C

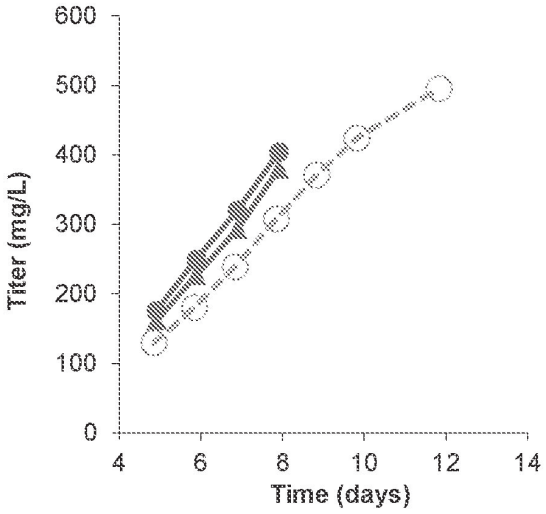
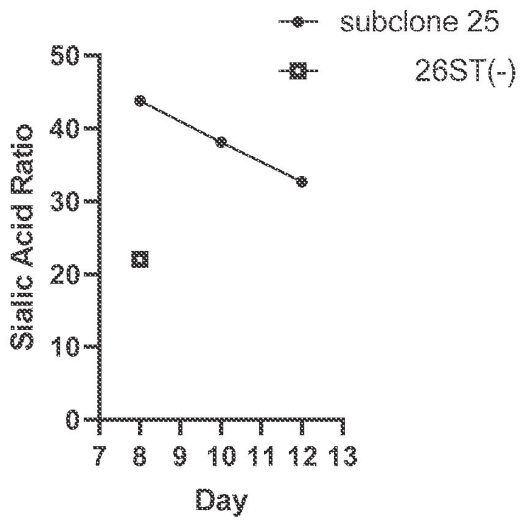



FIG. 9D




Anti-CD20 x CD3 IGM-A

FIG. 10A

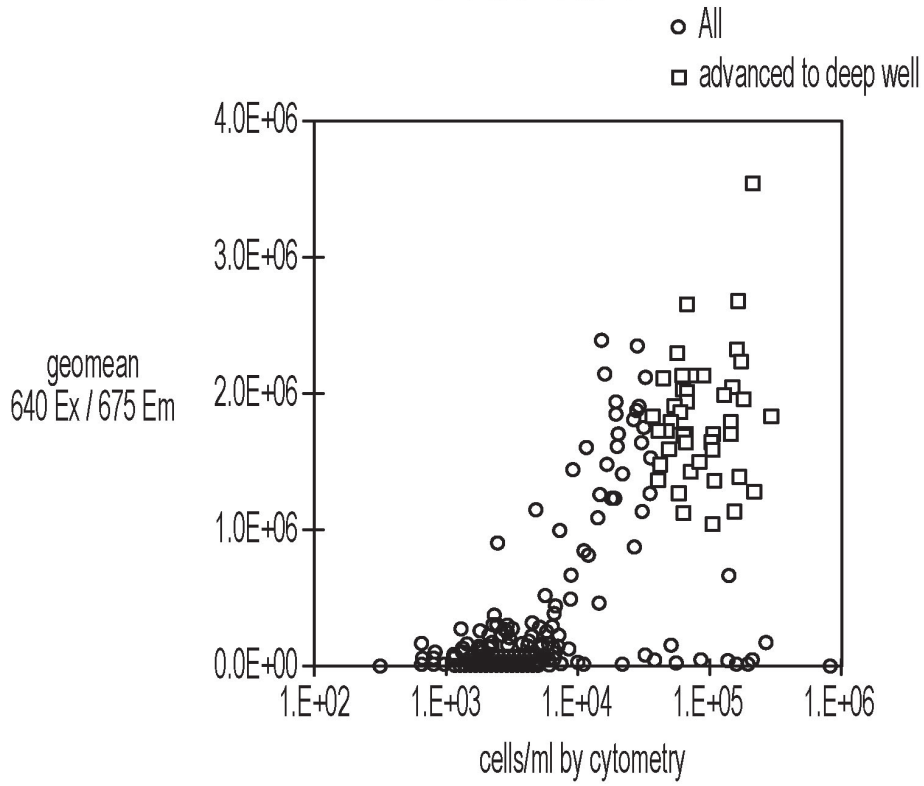



FIG. 10B

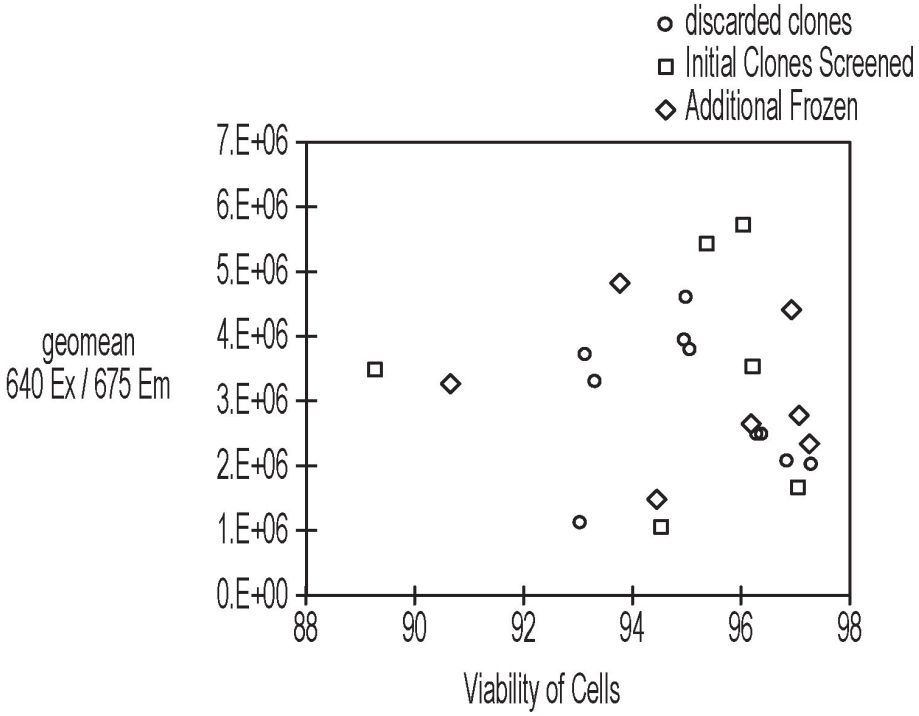


FIG. 11A

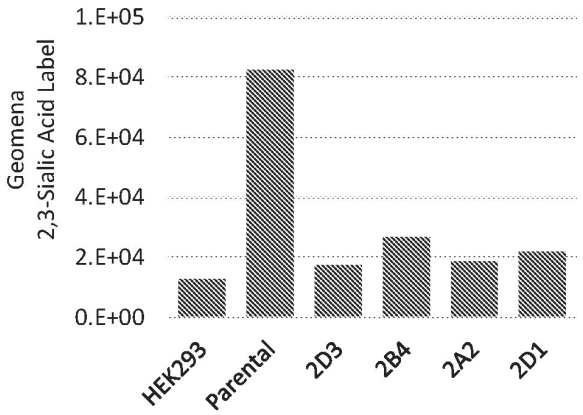



FIG. 11B


FIG. 11C

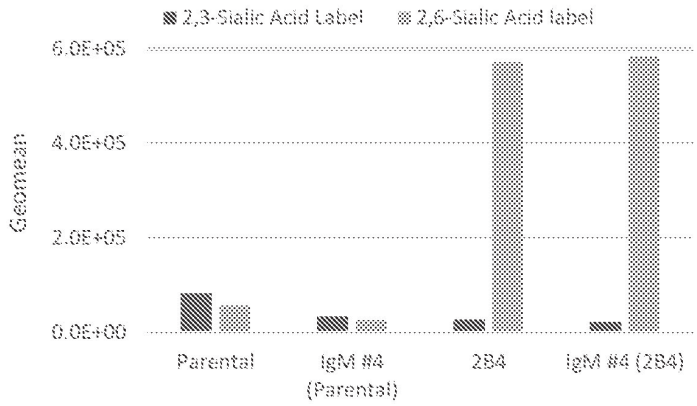


FIG. 12

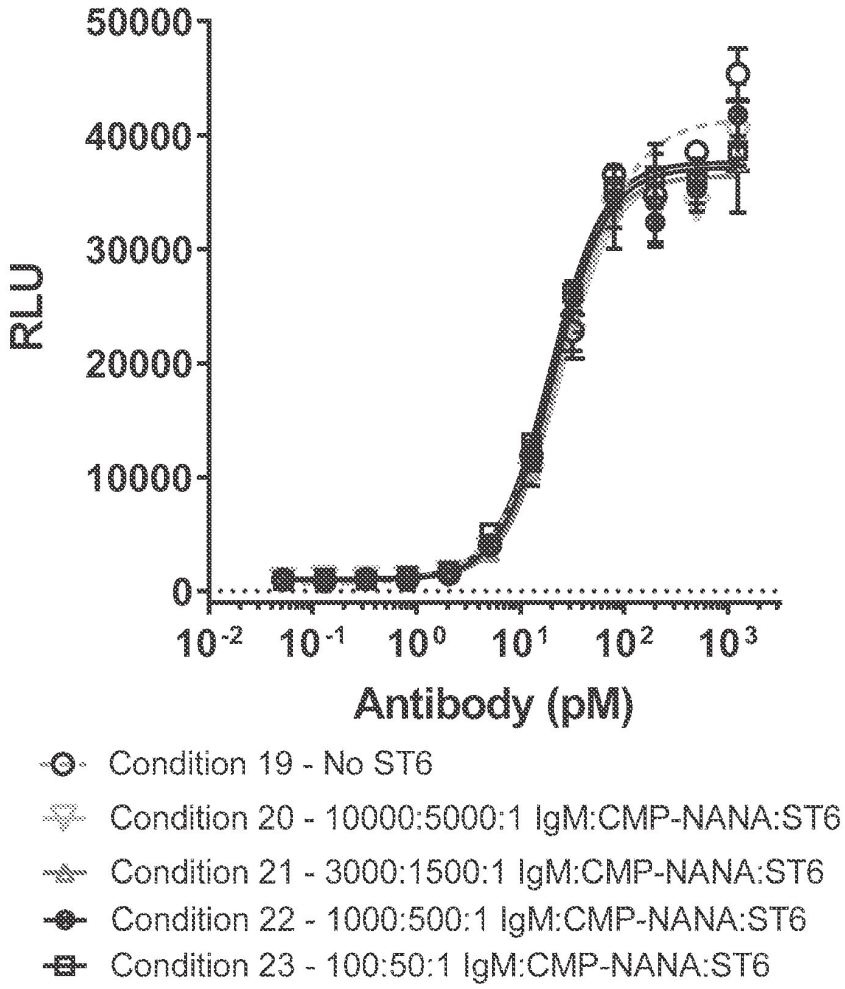
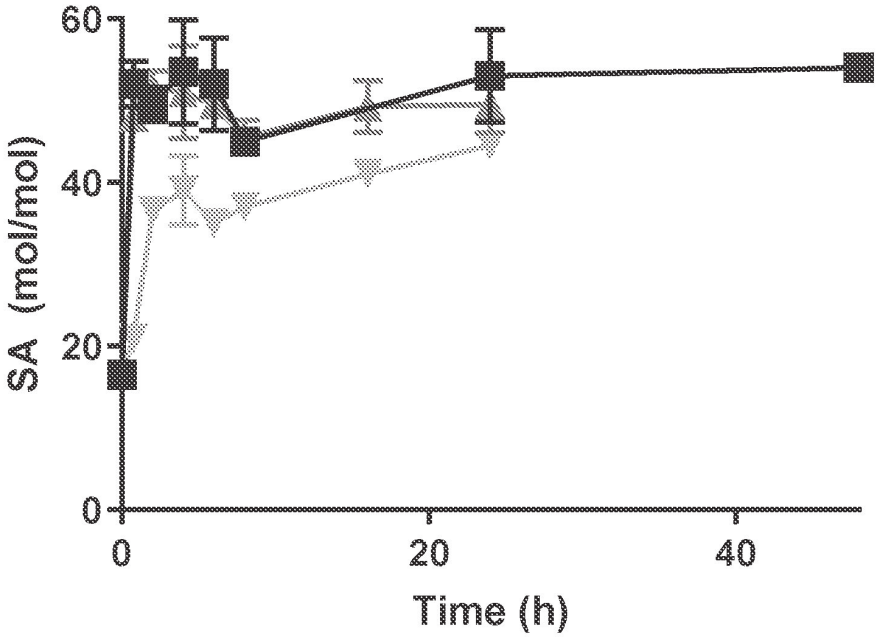

50000
40000
30000
20000
10000
0
RLU
10-2 10-1 100 101 102 103
Antibody (pM)
Condition 19 - No ST6
Condition 20 - 10000:5000:1 IgM:CMP-NANA:ST6
Condition 21 - 3000:1500:1 IgM:CMP-NANA:ST6
Condition 22 - 1000:500:1 IgM:CMP-NANA:ST6
Condition 23 - 100:50:1 IgM:CMP-NANA:ST6

FIG. 13A



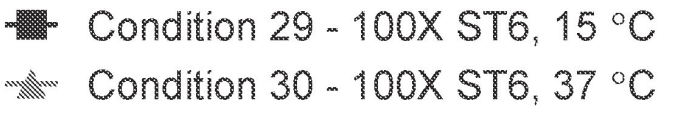




FIG. 13B

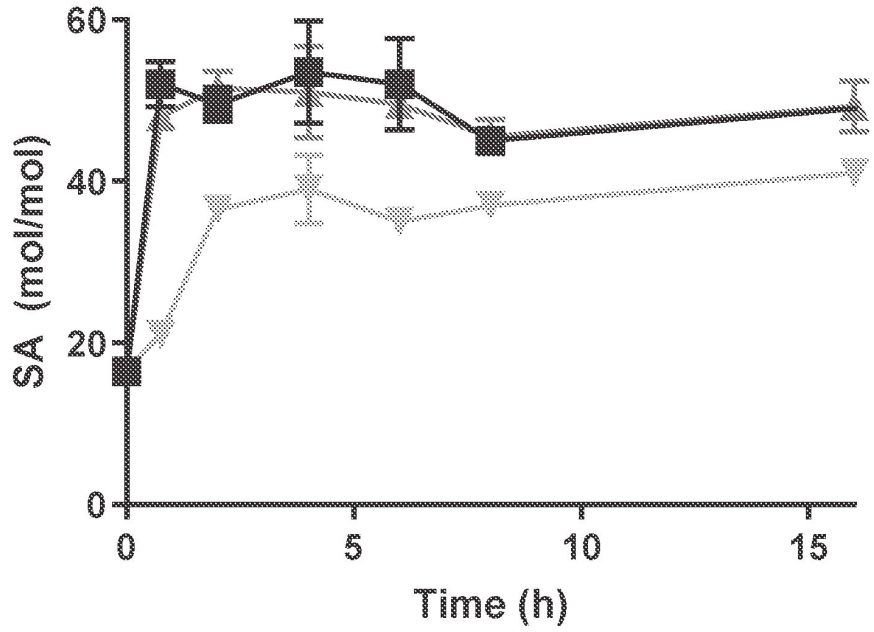


FIG. 14

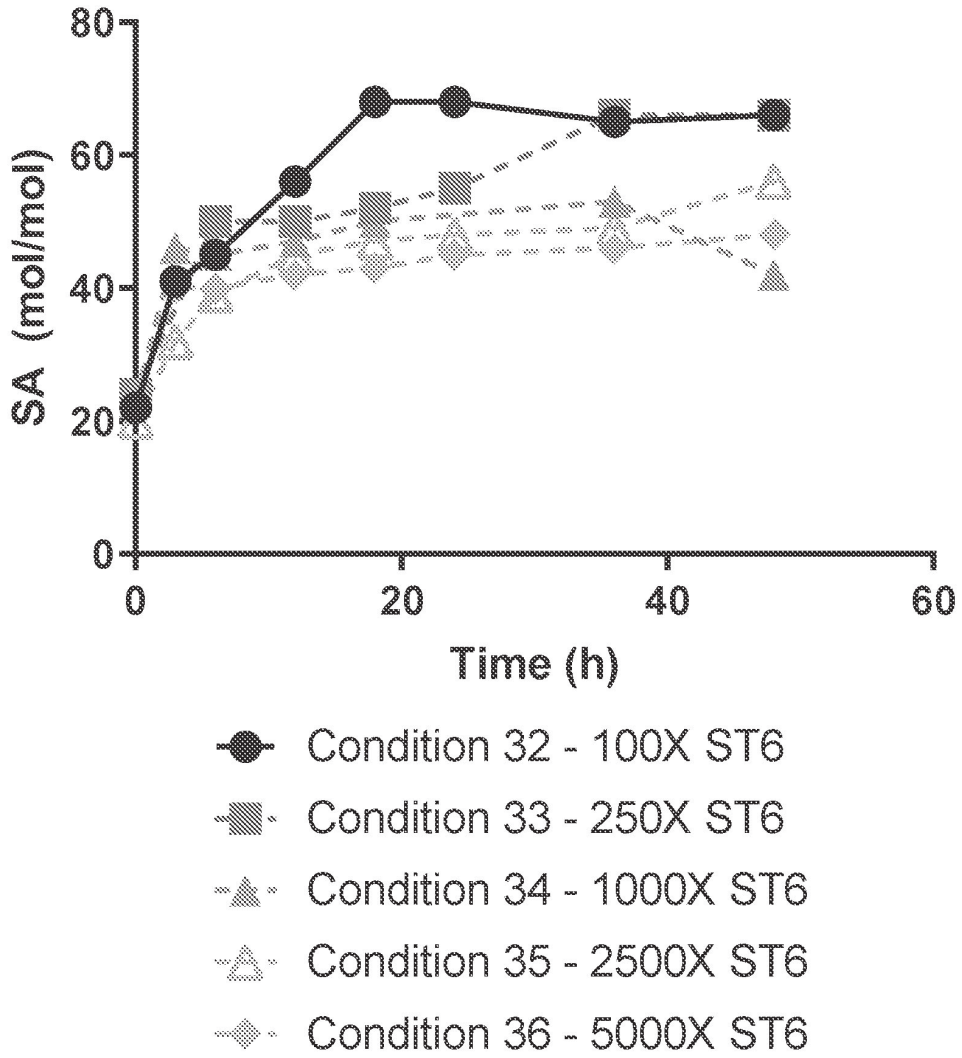

80
60
40
20
0
SA (mol/mol)
0
20
40
60
Time (h)
Condition 32 - 100X ST6
Condition 33 - 250X ST6
Condition 34 - 1000X ST6
Condition 35 - 2500X ST6
Condition 36 - 5000X ST6

FIG. 15

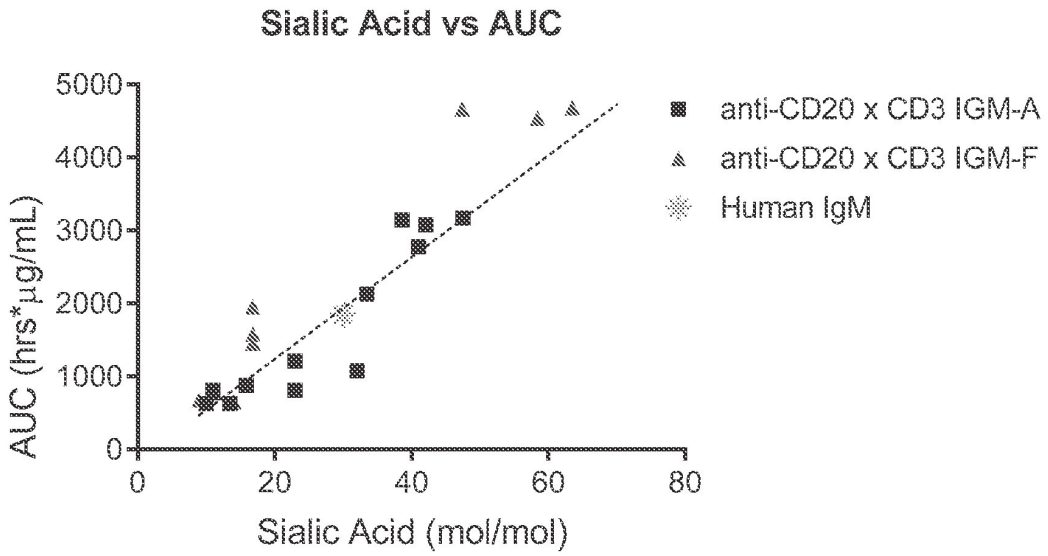

Sialic Acid vs AUC
AUC (hrs*µg/mL)
5000
4000
3000
2000
1000
0
0
20
40
60
80
Sialic Acid (mol/mol)
anti-CD20 x CD3 IGM-A
anti-CD20 x CD3 IGM-F
Human IgM

FIG. 16

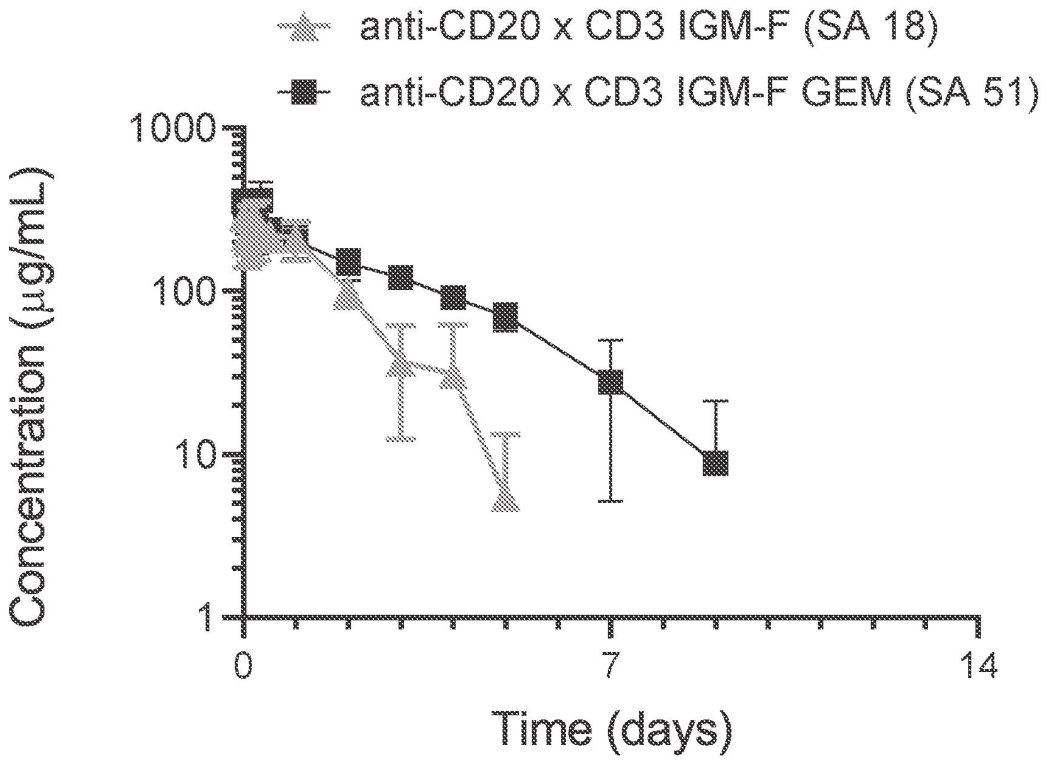

anti-CD20 x CD3 IGM-F (SA 18)
anti-CD20 x CD3 IGM-F GEM (SA 51)
Concentration (µg/mL)
1000
100
10
1
0
7
14
Time (days)

FIG. 17A

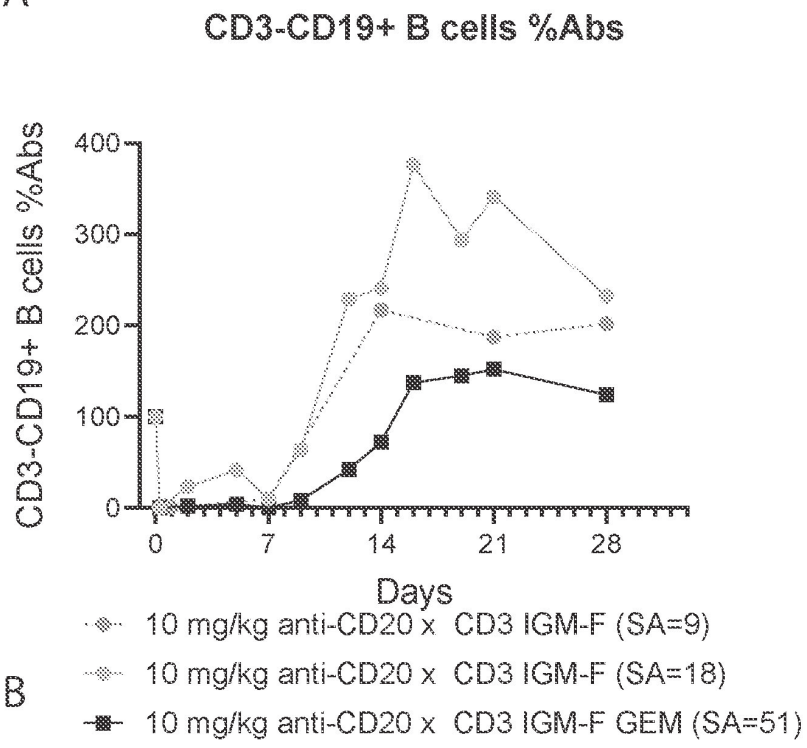
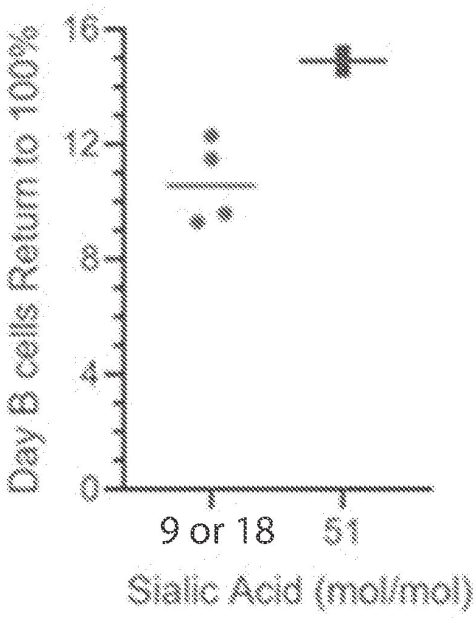



FIG. 17B



SEQUENCE LISTING

<110> IGM BIOSCIENCES, INC.

<120> HIGHLY SIALYLATED MULTIMERIC BINDING MOLECULES

<130> 004-028WO1

<140>
<141>

<150> 62/957,745
<151> 2020-01-06

<160> 48

<170> PatentIn version 3.5

<210> 1
<211> 453
<212> PRT
<213> Homo sapiens

<400> 1
Gly Ser Ala Ser Ala Pro Thr Leu Phe Pro Leu Val Ser Cys Glu Asn
1               5                  10                  15

Ser Pro Ser Asp Thr Ser Ser Val Ala Val Gly Cys Leu Ala Gln Asp
            20                  25                  30

Phe Leu Pro Asp Ser Ile Thr Phe Ser Trp Lys Tyr Lys Asn Asn Ser
        35                  40                  45

Asp Ile Ser Ser Thr Arg Gly Phe Pro Ser Val Leu Arg Gly Gly Lys
    50                  55                  60

Tyr Ala Ala Thr Ser Gln Val Leu Leu Pro Ser Lys Asp Val Met Gln
65                  70                  75                  80

Gly Thr Asp Glu His Val Val Cys Lys Val Gln His Pro Asn Gly Asn
                85                  90                  95

Lys Glu Lys Asn Val Pro Leu Pro Val Ile Ala Glu Leu Pro Pro Lys
            100                 105                 110

```
Val Ser Val Phe Val Pro Pro Arg Asp Gly Phe Phe Gly Asn Pro Arg
        115                 120                 125

Lys Ser Lys Leu Ile Cys Gln Ala Thr Gly Phe Ser Pro Arg Gln Ile
    130                 135                 140

Gln Val Ser Trp Leu Arg Glu Gly Lys Gln Val Gly Ser Gly Val Thr
145                 150                 155                 160

Thr Asp Gln Val Gln Ala Glu Ala Lys Glu Ser Gly Pro Thr Thr Tyr
                165                 170                 175

Lys Val Thr Ser Thr Leu Thr Ile Lys Glu Ser Asp Trp Leu Ser Gln
            180                 185                 190

Ser Met Phe Thr Cys Arg Val Asp His Arg Gly Leu Thr Phe Gln Gln
        195                 200                 205

Asn Ala Ser Ser Met Cys Val Pro Asp Gln Asp Thr Ala Ile Arg Val
    210                 215                 220

Phe Ala Ile Pro Pro Ser Phe Ala Ser Ile Phe Leu Thr Lys Ser Thr
225                 230                 235                 240

Lys Leu Thr Cys Leu Val Thr Asp Leu Thr Thr Tyr Asp Ser Val Thr
                245                 250                 255

Ile Ser Trp Thr Arg Gln Asn Gly Glu Ala Val Lys Thr His Thr Asn
            260                 265                 270

Ile Ser Glu Ser His Pro Asn Ala Thr Phe Ser Ala Val Gly Glu Ala
        275                 280                 285

Ser Ile Cys Glu Asp Asp Trp Asn Ser Gly Glu Arg Phe Thr Cys Thr
    290                 295                 300
```

```
Val Thr His Thr Asp Leu Pro Ser Pro Leu Lys Gln Thr Ile Ser Arg
305                 310                 315                 320


Pro Lys Gly Val Ala Leu His Arg Pro Asp Val Tyr Leu Leu Pro Pro
                325                 330                 335


Ala Arg Glu Gln Leu Asn Leu Arg Glu Ser Ala Thr Ile Thr Cys Leu
            340                 345                 350


Val Thr Gly Phe Ser Pro Ala Asp Val Phe Val Gln Trp Met Gln Arg
        355                 360                 365


Gly Gln Pro Leu Ser Pro Glu Lys Tyr Val Thr Ser Ala Pro Met Pro
    370                 375                 380


Glu Pro Gln Ala Pro Gly Arg Tyr Phe Ala His Ser Ile Leu Thr Val
385                 390                 395                 400


Ser Glu Glu Glu Trp Asn Thr Gly Glu Thr Tyr Thr Cys Val Val Ala
                405                 410                 415


His Glu Ala Leu Pro Asn Arg Val Thr Glu Arg Thr Val Asp Lys Ser
            420                 425                 430


Thr Gly Lys Pro Thr Leu Tyr Asn Val Ser Leu Val Met Ser Asp Thr
        435                 440                 445


Ala Gly Thr Cys Tyr
    450


<210> 2
<211> 453
<212> PRT
<213> Homo sapiens

<400> 2
Gly Ser Ala Ser Ala Pro Thr Leu Phe Pro Leu Val Ser Cys Glu Asn
1               5                   10                  15
```

```
Ser Pro Ser Asp Thr Ser Ser Val Ala Val Gly Cys Leu Ala Gln Asp
            20                  25                  30

Phe Leu Pro Asp Ser Ile Thr Phe Ser Trp Lys Tyr Lys Asn Asn Ser
        35                  40                  45

Asp Ile Ser Ser Thr Arg Gly Phe Pro Ser Val Leu Arg Gly Gly Lys
    50                  55                  60

Tyr Ala Ala Thr Ser Gln Val Leu Leu Pro Ser Lys Asp Val Met Gln
65                  70                  75                  80

Gly Thr Asp Glu His Val Val Cys Lys Val Gln His Pro Asn Gly Asn
                85                  90                  95

Lys Glu Lys Asn Val Pro Leu Pro Val Ile Ala Glu Leu Pro Pro Lys
            100                 105                 110

Val Ser Val Phe Val Pro Pro Arg Asp Gly Phe Phe Gly Asn Pro Arg
        115                 120                 125

Lys Ser Lys Leu Ile Cys Gln Ala Thr Gly Phe Ser Pro Arg Gln Ile
    130                 135                 140

Gln Val Ser Trp Leu Arg Glu Gly Lys Gln Val Gly Ser Gly Val Thr
145                 150                 155                 160

Thr Asp Gln Val Gln Ala Glu Ala Lys Glu Ser Gly Pro Thr Thr Tyr
                165                 170                 175

Lys Val Thr Ser Thr Leu Thr Ile Lys Glu Ser Asp Trp Leu Gly Gln
            180                 185                 190

Ser Met Phe Thr Cys Arg Val Asp His Arg Gly Leu Thr Phe Gln Gln
        195                 200                 205

Asn Ala Ser Ser Met Cys Val Pro Asp Gln Asp Thr Ala Ile Arg Val
    210                 215                 220
```

```
Phe Ala Ile Pro Pro Ser Phe Ala Ser Ile Phe Leu Thr Lys Ser Thr
225                 230                 235                 240

Lys Leu Thr Cys Leu Val Thr Asp Leu Thr Thr Tyr Asp Ser Val Thr
                245                 250                 255

Ile Ser Trp Thr Arg Gln Asn Gly Glu Ala Val Lys Thr His Thr Asn
            260                 265                 270

Ile Ser Glu Ser His Pro Asn Ala Thr Phe Ser Ala Val Gly Glu Ala
        275                 280                 285

Ser Ile Cys Glu Asp Asp Trp Asn Ser Gly Glu Arg Phe Thr Cys Thr
    290                 295                 300

Val Thr His Thr Asp Leu Pro Ser Pro Leu Lys Gln Thr Ile Ser Arg
305                 310                 315                 320

Pro Lys Gly Val Ala Leu His Arg Pro Asp Val Tyr Leu Leu Pro Pro
                325                 330                 335

Ala Arg Glu Gln Leu Asn Leu Arg Glu Ser Ala Thr Ile Thr Cys Leu
            340                 345                 350

Val Thr Gly Phe Ser Pro Ala Asp Val Phe Val Gln Trp Met Gln Arg
        355                 360                 365

Gly Gln Pro Leu Ser Pro Glu Lys Tyr Val Thr Ser Ala Pro Met Pro
    370                 375                 380

Glu Pro Gln Ala Pro Gly Arg Tyr Phe Ala His Ser Ile Leu Thr Val
385                 390                 395                 400

Ser Glu Glu Glu Trp Asn Thr Gly Glu Thr Tyr Thr Cys Val Val Ala
                405                 410                 415
```

```
His Glu Ala Leu Pro Asn Arg Val Thr Glu Arg Thr Val Asp Lys Ser
            420                 425                 430


Thr Gly Lys Pro Thr Leu Tyr Asn Val Ser Leu Val Met Ser Asp Thr
        435                 440                 445


Ala Gly Thr Cys Tyr
    450


<210> 3
<211> 406
<212> PRT
<213> Homo sapiens

<400> 3
Met Ile His Thr Asn Leu Lys Lys Lys Phe Ser Cys Cys Val Leu Val
1               5                   10                  15


Phe Leu Leu Phe Ala Val Ile Cys Val Trp Lys Glu Lys Lys Lys Gly
            20                  25                  30


Ser Tyr Tyr Asp Ser Phe Lys Leu Gln Thr Lys Glu Phe Gln Val Leu
        35                  40                  45


Lys Ser Leu Gly Lys Leu Ala Met Gly Ser Asp Ser Gln Ser Val Ser
    50                  55                  60


Ser Ser Ser Thr Gln Asp Pro His Arg Gly Arg Gln Thr Leu Gly Ser
65                  70                  75                  80


Leu Arg Gly Leu Ala Lys Ala Lys Pro Glu Ala Ser Phe Gln Val Trp
                85                  90                  95


Asn Lys Asp Ser Ser Ser Lys Asn Leu Ile Pro Arg Leu Gln Lys Ile
            100                 105                 110


Trp Lys Asn Tyr Leu Ser Met Asn Lys Tyr Lys Val Ser Tyr Lys Gly
        115                 120                 125
```

```
Pro Gly Pro Gly Ile Lys Phe Ser Ala Glu Ala Leu Arg Cys His Leu
    130                 135                 140

Arg Asp His Val Asn Val Ser Met Val Glu Val Thr Asp Phe Pro Phe
145                 150                 155                 160

Asn Thr Ser Glu Trp Glu Gly Tyr Leu Pro Lys Glu Ser Ile Arg Thr
                165                 170                 175

Lys Ala Gly Pro Trp Gly Arg Cys Ala Val Val Ser Ser Ala Gly Ser
            180                 185                 190

Leu Lys Ser Ser Gln Leu Gly Arg Glu Ile Asp Asp His Asp Ala Val
        195                 200                 205

Leu Arg Phe Asn Gly Ala Pro Thr Ala Asn Phe Gln Gln Asp Val Gly
    210                 215                 220

Thr Lys Thr Thr Ile Arg Leu Met Asn Ser Gln Leu Val Thr Thr Glu
225                 230                 235                 240

Lys Arg Phe Leu Lys Asp Ser Leu Tyr Asn Glu Gly Ile Leu Ile Val
                245                 250                 255

Trp Asp Pro Ser Val Tyr His Ser Asp Ile Pro Lys Trp Tyr Gln Asn
            260                 265                 270

Pro Asp Tyr Asn Phe Phe Asn Asn Tyr Lys Thr Tyr Arg Lys Leu His
        275                 280                 285

Pro Asn Gln Pro Phe Tyr Ile Leu Lys Pro Gln Met Pro Trp Glu Leu
    290                 295                 300

Trp Asp Ile Leu Gln Glu Ile Ser Pro Glu Glu Ile Gln Pro Asn Pro
305                 310                 315                 320

Pro Ser Ser Gly Met Leu Gly Ile Ile Ile Met Met Thr Leu Cys Asp
                325                 330                 335
```

```
Gln Val Asp Ile Tyr Glu Phe Leu Pro Ser Lys Arg Lys Thr Asp Val
            340                 345                 350


Cys Tyr Tyr Tyr Gln Lys Phe Phe Asp Ser Ala Cys Thr Met Gly Ala
        355                 360                 365


Tyr His Pro Leu Leu Tyr Glu Lys Asn Leu Val Lys His Leu Asn Gln
    370                 375                 380


Gly Thr Asp Glu Asp Ile Tyr Leu Leu Gly Lys Ala Thr Leu Pro Gly
385                 390                 395                 400


Phe Arg Thr Ile His Cys
                405


<210> 4
<211> 344
<212> PRT
<213> Homo sapiens

<400> 4
Met Gly Phe Asn Leu Thr Phe His Leu Ser Tyr Lys Phe Arg Leu Leu
1               5                   10                  15


Leu Leu Leu Thr Leu Cys Leu Thr Val Val Gly Trp Ala Thr Ser Asn
            20                  25                  30


Tyr Phe Val Gly Ala Ile Gln Glu Ile Pro Lys Ala Lys Glu Phe Met
        35                  40                  45


Ala Asn Phe His Lys Thr Leu Ile Leu Gly Lys Gly Lys Thr Leu Thr
    50                  55                  60


Asn Glu Ala Ser Thr Lys Lys Val Glu Leu Asp Asn Cys Pro Ser Val
65                  70                  75                  80


Ser Pro Tyr Leu Arg Gly Gln Ser Lys Leu Ile Phe Lys Pro Asp Leu
                85                  90                  95
```

```
Thr Leu Glu Glu Val Gln Ala Glu Asn Pro Lys Val Ser Arg Gly Arg
            100                 105                 110

Tyr Arg Pro Gln Glu Cys Lys Ala Leu Gln Arg Val Ala Ile Leu Val
        115                 120                 125

Pro His Arg Asn Arg Glu Lys His Leu Met Tyr Leu Leu Glu His Leu
    130                 135                 140

His Pro Phe Leu Gln Arg Gln Gln Leu Asp Tyr Gly Ile Tyr Val Ile
145                 150                 155                 160

His Gln Ala Glu Gly Lys Lys Phe Asn Arg Ala Lys Leu Leu Asn Val
                165                 170                 175

Gly Tyr Leu Glu Ala Leu Lys Glu Glu Asn Trp Asp Cys Phe Ile Phe
            180                 185                 190

His Asp Val Asp Leu Val Pro Glu Asn Asp Phe Asn Leu Tyr Lys Cys
        195                 200                 205

Glu Glu His Pro Lys His Leu Val Val Gly Arg Asn Ser Thr Gly Tyr
    210                 215                 220

Arg Leu Arg Tyr Ser Gly Tyr Phe Gly Gly Val Thr Ala Leu Ser Arg
225                 230                 235                 240

Glu Gln Phe Phe Lys Val Asn Gly Phe Ser Asn Asn Tyr Trp Gly Trp
                245                 250                 255

Gly Gly Glu Asp Asp Asp Leu Arg Leu Arg Val Glu Leu Gln Arg Met
            260                 265                 270

Lys Ile Ser Arg Pro Leu Pro Glu Val Gly Lys Tyr Thr Met Val Phe
        275                 280                 285
```

```
His Thr Arg Asp Lys Gly Asn Glu Val Asn Ala Glu Arg Met Lys Leu
    290                 295                 300

Leu His Gln Val Ser Arg Val Trp Arg Thr Asp Gly Leu Ser Ser Cys
305                 310                 315                 320

Ser Tyr Lys Leu Val Ser Val Glu His Asn Pro Leu Tyr Ile Asn Ile
                325                 330                 335

Thr Val Asp Phe Trp Phe Gly Ala
            340
```

<210> 5
<211> 159
<212> PRT
<213> Homo sapiens

<400> 5

```
Met Lys Asn His Leu Leu Phe Trp Gly Val Leu Ala Val Phe Ile Lys
1               5                   10                  15

Ala Val His Val Lys Ala Gln Glu Asp Glu Arg Ile Val Leu Val Asp
            20                  25                  30

Asn Lys Cys Lys Cys Ala Arg Ile Thr Ser Arg Ile Ile Arg Ser Ser
        35                  40                  45

Glu Asp Pro Asn Glu Asp Ile Val Glu Arg Asn Ile Arg Ile Ile Val
    50                  55                  60

Pro Leu Asn Asn Arg Glu Asn Ile Ser Asp Pro Thr Ser Pro Leu Arg
65                  70                  75                  80

Thr Arg Phe Val Tyr His Leu Ser Asp Leu Cys Lys Lys Cys Asp Pro
                85                  90                  95

Thr Glu Val Glu Leu Asp Asn Gln Ile Val Thr Ala Thr Gln Ser Asn
            100                 105                 110
```

```
Ile Cys Asp Glu Asp Ser Ala Thr Glu Thr Cys Tyr Thr Tyr Asp Arg
        115                 120                 125


Asn Lys Cys Tyr Thr Ala Val Val Pro Leu Val Tyr Gly Gly Glu Thr
    130                 135                 140


Lys Met Val Glu Thr Ala Leu Thr Pro Asp Ala Cys Tyr Pro Asp
145                 150                 155


<210> 6
<211> 137
<212> PRT
<213> Homo sapiens

<400> 6
Gln Glu Asp Glu Arg Ile Val Leu Val Asp Asn Lys Cys Lys Cys Ala
1               5                   10                  15


Arg Ile Thr Ser Arg Ile Ile Arg Ser Ser Glu Asp Pro Asn Glu Asp
            20                  25                  30


Ile Val Glu Arg Asn Ile Arg Ile Ile Val Pro Leu Asn Asn Arg Glu
        35                  40                  45


Asn Ile Ser Asp Pro Thr Ser Pro Leu Arg Thr Arg Phe Val Tyr His
    50                  55                  60


Leu Ser Asp Leu Cys Lys Lys Cys Asp Pro Thr Glu Val Glu Leu Asp
65                  70                  75                  80


Asn Gln Ile Val Thr Ala Thr Gln Ser Asn Ile Cys Asp Glu Asp Ser
                85                  90                  95


Ala Thr Glu Thr Cys Tyr Thr Tyr Asp Arg Asn Lys Cys Tyr Thr Ala
            100                 105                 110


Val Val Pro Leu Val Tyr Gly Gly Glu Thr Lys Met Val Glu Thr Ala
        115                 120                 125
```

Leu Thr Pro Asp Ala Cys Tyr Pro Asp
130 135

<210> 7
<211> 137
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 7
Gln Glu Asp Glu Arg Ile Val Leu Val Asp Asn Lys Cys Lys Cys Ala
1 5 10 15

Arg Ile Thr Ser Arg Ile Ile Arg Ser Ser Glu Asp Pro Asn Glu Asp
20 25 30

Ile Val Glu Arg Asn Ile Arg Ile Ile Val Pro Leu Asn Asn Arg Glu
35 40 45

Asn Ile Ser Asp Pro Thr Ser Pro Leu Arg Thr Arg Phe Val Tyr His
50 55 60

Leu Ser Asp Leu Cys Lys Lys Cys Asp Pro Thr Glu Val Glu Leu Asp
65 70 75 80

Asn Gln Ile Val Thr Ala Thr Gln Ser Asn Ile Cys Asp Glu Asp Ser
85 90 95

Ala Thr Glu Thr Cys Ala Thr Tyr Asp Arg Asn Lys Cys Tyr Thr Ala
100 105 110

Val Val Pro Leu Val Tyr Gly Gly Glu Thr Lys Met Val Glu Thr Ala
115 120 125

Leu Thr Pro Asp Ala Cys Tyr Pro Asp
130 135

<210> 8
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 8
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1 5 10 15

Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Ser Tyr
20 25 30

Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
50 55 60

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Thr Thr Ala Tyr
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg His Pro Ser Tyr Gly Ser Gly Ser Pro Asn Phe Asp Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 9
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 9
Asp Ile Val Met Thr Gln Thr Pro Leu Ser Ser Pro Val Thr Leu Gly
1               5                  10                  15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val Tyr Ser
            20                  25                  30

Asp Gly Asn Thr Tyr Leu Ser Trp Leu Gln Gln Arg Pro Gly Gln Pro
        35                  40                  45

Pro Arg Leu Leu Ile Tyr Lys Ile Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ala Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Val Gln Ala
                85                  90                  95

Thr Gln Phe Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

<210> 10
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 10
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Val Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Thr Ile Ser Ser Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg Gly Asp Ser Met Ile Thr Thr Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser Ala
        115                 120

<210> 11
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 11
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Gly Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Trp Ala Ser Thr Arg His Thr Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Ser Tyr Arg Thr
85 90 95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 12
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 12
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Gly
20 25 30

Asp Tyr Phe Trp Ser Trp Ile Arg Gln Leu Pro Gly Lys Gly Leu Glu
35 40 45

Cys Ile Gly His Ile His Asn Ser Gly Thr Thr Tyr Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Lys Gln Phe
65 70 75 80

Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Ala Arg Asp Arg Gly Gly Asp Tyr Tyr Tyr Gly Met Asp Val Trp
100 105 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 13
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 13
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Gly Ile Ser Arg Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Ser Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Phe Gly Ser Ser Pro
85 90 95

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 14
<211> 125
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 14

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Lys Gly
1               5                   10                  15


Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
            20                  25                  30


Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60


Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Gln Ser Ile
65                  70                  75                  80


Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr
                85                  90                  95


Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
            100                 105                 110


Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120                 125


<210> 15
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<400> 15
Gly Phe Thr Phe Asn Thr Tyr Ala Met Asn
1               5                   10


<210> 16
<211> 20
<212> PRT
<213> Artificial Sequence
```

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 16
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
1 5 10 15

Ser Val Lys Asp
20

<210> 17
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 17
Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe Ala Tyr
1 5 10 15

<210> 18
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 18
Gln Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu
1 5 10 15

Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30

Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly
35 40 45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
    50                  55                  60

Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala
65                  70                  75                  80

Gln Thr Glu Asp Glu Ala Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn
                85                  90                  95

Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105

<210> 19
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<400> 19
Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
1               5                   10

<210> 20
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<400> 20
Gly Thr Asn Lys Arg Ala Pro
1               5

<210> 21
<211> 7
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
peptide

<400> 21
Ala Leu Trp Tyr Ser Asn Leu
1 5

<210> 22
<211> 125
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 22
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Thr Tyr
20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Thr
65 70 75 80

Leu Tyr Leu Gln Met Glu Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Val Arg His Gly Asn Phe Gln Gly Gly Tyr Val Ser Trp Phe
100 105 110

Ala His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 23
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 23
Gln Thr Val Val Thr Gln Glu Pro Ser Leu Ser Val Ser Pro Gly Gly
1 5 10 15

Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30

Asn Tyr Ala Asn Trp Val Gln Gln Thr Pro Gly Gln Ala Pro Arg Gly
35 40 45

Leu Ile Gly Gly Thr Asp Lys Arg Ala Pro Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Leu Leu Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala
65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
85 90 95

His Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> 24
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 24
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

```
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Thr Tyr
            20                  25                  30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Thr
65                  70                  75                  80

Leu Tyr Leu Gln Met Glu Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95

Tyr Cys Val Arg His Gly Asn Phe Gln Gly Gly Tyr Val Ser Trp Phe
            100                 105                 110

Ala His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Thr Val Val
    130                 135                 140

Thr Gln Glu Pro Ser Leu Ser Val Ser Pro Gly Gly Thr Val Thr Leu
145                 150                 155                 160

Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
                165                 170                 175

Trp Val Gln Gln Thr Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly
            180                 185                 190

Thr Asp Lys Arg Ala Pro Gly Val Pro Asp Arg Phe Ser Gly Ser Leu
        195                 200                 205
```

Leu Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Ala Glu Asp
210 215 220

Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn His Trp Val Phe
225 230 235 240

Gly Gly Gly Thr Lys Leu Thr Val Leu
245

<210> 25
<211> 125
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 25
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Thr Tyr
20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Thr
65 70 75 80

Leu Tyr Leu Gln Met Glu Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Val Arg His Gly Asn Phe Gly Gly Gly Tyr Val Ser Trp Phe
100 105 110

Ala Trp Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120                 125


<210> 26
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 26
Gln Thr Val Val Thr Gln Glu Pro Ser Leu Ser Val Ser Pro Gly Gly
1               5                   10                  15


Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
            20                  25                  30


Asn Tyr Ala Asn Trp Val Gln Gln Thr Pro Gly Gln Ala Pro Arg Gly
        35                  40                  45


Leu Ile Gly Gly Thr Asp Lys Arg Ala Pro Gly Val Pro Asp Arg Phe
    50                  55                  60


Ser Gly Ser Leu Leu Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala
65                  70                  75                  80


Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
                85                  90                  95


His Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105


<210> 27
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 27
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Thr Tyr
            20                  25                  30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Thr
65                  70                  75                  80

Leu Tyr Leu Gln Met Glu Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95

Tyr Cys Val Arg His Gly Asn Phe Gly Gly Gly Tyr Val Ser Trp Phe
            100                 105                 110

Ala Trp Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Thr Val Val
    130                 135                 140

Thr Gln Glu Pro Ser Leu Ser Val Ser Pro Gly Gly Thr Val Thr Leu
145                 150                 155                 160

Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
                165                 170                 175

Trp Val Gln Gln Thr Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly
            180                 185                 190

Thr Asp Lys Arg Ala Pro Gly Val Pro Asp Arg Phe Ser Gly Ser Leu
195 200 205

Leu Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Ala Glu Asp
210 215 220

Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn His Trp Val Phe
225 230 235 240

Gly Gly Gly Thr Lys Leu Thr Val Leu
245

<210> 28
<211> 125
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 28
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Thr Tyr
20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Thr
65 70 75 80

Leu Tyr Leu Gln Met Glu Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Val Arg His Ala Asn Phe Gly Ala Gly Tyr Val Ser Trp Phe
100 105 110

Ala His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 29
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 29
Gln Thr Val Val Thr Gln Glu Pro Ser Leu Ser Val Ser Pro Gly Gly
1 5 10 15

Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30

Asn Tyr Ala Asn Trp Val Gln Gln Thr Pro Gly Gln Ala Pro Arg Gly
35 40 45

Leu Ile Gly Gly Thr Asp Lys Arg Ala Pro Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Leu Leu Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala
65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
85 90 95

His Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> 30
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 30
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Thr Tyr
20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Thr
65 70 75 80

Leu Tyr Leu Gln Met Glu Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Val Arg His Ala Asn Phe Gly Ala Gly Tyr Val Ser Trp Phe
100 105 110

Ala His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Thr Val Val
130 135 140

Thr Gln Glu Pro Ser Leu Ser Val Ser Pro Gly Gly Thr Val Thr Leu
145 150 155 160

Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
165 170 175

```
Trp Val Gln Gln Thr Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly
            180                 185                 190


Thr Asp Lys Arg Ala Pro Gly Val Pro Asp Arg Phe Ser Gly Ser Leu
        195                 200                 205


Leu Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Ala Glu Asp
    210                 215                 220


Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn His Trp Val Phe
225                 230                 235                 240


Gly Gly Gly Thr Lys Leu Thr Val Leu
                245
```

<210> 31
<211> 401
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 31

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Thr Tyr
            20                  25                  30


Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60


Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Thr
65                  70                  75                  80
```

```
Leu Tyr Leu Gln Met Glu Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95

Tyr Cys Val Arg His Gly Asn Phe Gln Gly Gly Tyr Val Ser Trp Phe
            100                 105                 110

Ala His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Thr Val Val
    130                 135                 140

Thr Gln Glu Pro Ser Leu Ser Val Ser Pro Gly Gly Thr Val Thr Leu
145                 150                 155                 160

Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
                165                 170                 175

Trp Val Gln Gln Thr Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly
            180                 185                 190

Thr Asp Lys Arg Ala Pro Gly Val Pro Asp Arg Phe Ser Gly Ser Leu
        195                 200                 205

Leu Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Ala Glu Asp
    210                 215                 220

Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn His Trp Val Phe
225                 230                 235                 240

Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gly Gly Gly Ser Gly Gly
                245                 250                 255

Gly Gly Ser Gly Gly Gly Gly Ser Gln Glu Asp Glu Arg Ile Val Leu
            260                 265                 270

Val Asp Asn Lys Cys Lys Cys Ala Arg Ile Thr Ser Arg Ile Ile Arg
        275                 280                 285
```

Ser Ser Glu Asp Pro Asn Glu Asp Ile Val Glu Arg Asn Ile Arg Ile
290 295 300

Ile Val Pro Leu Asn Asn Arg Glu Asn Ile Ser Asp Pro Thr Ser Pro
305 310 315 320

Leu Arg Thr Arg Phe Val Tyr His Leu Ser Asp Leu Cys Lys Lys Cys
325 330 335

Asp Pro Thr Glu Val Glu Leu Asp Asn Gln Ile Val Thr Ala Thr Gln
340 345 350

Ser Asn Ile Cys Asp Glu Asp Ser Ala Thr Glu Thr Cys Ala Thr Tyr
355 360 365

Asp Arg Asn Lys Cys Tyr Thr Ala Val Val Pro Leu Val Tyr Gly Gly
370 375 380

Glu Thr Lys Met Val Glu Thr Ala Leu Thr Pro Asp Ala Cys Tyr Pro
385 390 395 400

Asp

<210> 32
<211> 401
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 32
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Thr Tyr
20 25 30

```
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
    50                  55                  60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Thr
65                  70                  75                  80

Leu Tyr Leu Gln Met Glu Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95

Tyr Cys Val Arg His Gly Asn Phe Gly Gly Gly Tyr Val Ser Trp Phe
            100                 105                 110

Ala Trp Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Thr Val Val
    130                 135                 140

Thr Gln Glu Pro Ser Leu Ser Val Ser Pro Gly Gly Thr Val Thr Leu
145                 150                 155                 160

Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
                165                 170                 175

Trp Val Gln Gln Thr Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly
            180                 185                 190

Thr Asp Lys Arg Ala Pro Gly Val Pro Asp Arg Phe Ser Gly Ser Leu
        195                 200                 205

Leu Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Ala Glu Asp
    210                 215                 220
```

```
Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn His Trp Val Phe
225                 230                 235                 240

Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gly Gly Gly Ser Gly Gly
                245                 250                 255

Gly Gly Ser Gly Gly Gly Gly Ser Gln Glu Asp Glu Arg Ile Val Leu
            260                 265                 270

Val Asp Asn Lys Cys Lys Cys Ala Arg Ile Thr Ser Arg Ile Ile Arg
        275                 280                 285

Ser Ser Glu Asp Pro Asn Glu Asp Ile Val Glu Arg Asn Ile Arg Ile
    290                 295                 300

Ile Val Pro Leu Asn Asn Arg Glu Asn Ile Ser Asp Pro Thr Ser Pro
305                 310                 315                 320

Leu Arg Thr Arg Phe Val Tyr His Leu Ser Asp Leu Cys Lys Lys Cys
                325                 330                 335

Asp Pro Thr Glu Val Glu Leu Asp Asn Gln Ile Val Thr Ala Thr Gln
            340                 345                 350

Ser Asn Ile Cys Asp Glu Asp Ser Ala Thr Glu Thr Cys Ala Thr Tyr
        355                 360                 365

Asp Arg Asn Lys Cys Tyr Thr Ala Val Val Pro Leu Val Tyr Gly Gly
    370                 375                 380

Glu Thr Lys Met Val Glu Thr Ala Leu Thr Pro Asp Ala Cys Tyr Pro
385                 390                 395                 400

Asp
```

<210> 33
<211> 401

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 33
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Thr Tyr
20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Thr
65 70 75 80

Leu Tyr Leu Gln Met Glu Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Val Arg His Ala Asn Phe Gly Ala Gly Tyr Val Ser Trp Phe
100 105 110

Ala His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Thr Val Val
130 135 140

Thr Gln Glu Pro Ser Leu Ser Val Ser Pro Gly Gly Thr Val Thr Leu
145 150 155 160

Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
165 170 175

```
Trp Val Gln Gln Thr Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly
            180                 185                 190

Thr Asp Lys Arg Ala Pro Gly Val Pro Asp Arg Phe Ser Gly Ser Leu
        195                 200                 205

Leu Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Ala Glu Asp
    210                 215                 220

Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn His Trp Val Phe
225                 230                 235                 240

Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gly Gly Gly Ser Gly Gly
                245                 250                 255

Gly Gly Ser Gly Gly Gly Gly Ser Gln Glu Asp Glu Arg Ile Val Leu
            260                 265                 270

Val Asp Asn Lys Cys Lys Cys Ala Arg Ile Thr Ser Arg Ile Ile Arg
        275                 280                 285

Ser Ser Glu Asp Pro Asn Glu Asp Ile Val Glu Arg Asn Ile Arg Ile
    290                 295                 300

Ile Val Pro Leu Asn Asn Arg Glu Asn Ile Ser Asp Pro Thr Ser Pro
305                 310                 315                 320

Leu Arg Thr Arg Phe Val Tyr His Leu Ser Asp Leu Cys Lys Lys Cys
                325                 330                 335

Asp Pro Thr Glu Val Glu Leu Asp Asn Gln Ile Val Thr Ala Thr Gln
            340                 345                 350

Ser Asn Ile Cys Asp Glu Asp Ser Ala Thr Glu Thr Cys Ala Thr Tyr
        355                 360                 365
```

Asp Arg Asn Lys Cys Tyr Thr Ala Val Val Pro Leu Val Tyr Gly Gly
370 375 380

Glu Thr Lys Met Val Glu Thr Ala Leu Thr Pro Asp Ala Cys Tyr Pro
385 390 395 400

Asp

<210> 34
<211> 1012
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 34
Met Gly Trp Ser Tyr Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Ile Ser Tyr Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Glu Trp Met Gly Tyr Ile Asn Pro Arg Ser Gly Tyr Thr His Tyr Asn
65 70 75 80

Gln Lys Leu Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ala Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

```
Tyr Tyr Cys Ala Arg Ser Ala Tyr Tyr Asp Tyr Asp Gly Phe Ala Tyr
        115                 120                 125

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser
    130                 135                 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln
145                 150                 155                 160

Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr
                165                 170                 175

Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys
            180                 185                 190

Pro Gly Lys Ala Pro Lys Arg Leu Ile Tyr Asp Thr Ser Lys Leu Ala
        195                 200                 205

Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
    210                 215                 220

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
225                 230                 235                 240

Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys
                245                 250                 255

Val Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
            260                 265                 270

Gly Gly Ser Gln Glu Asp Glu Arg Ile Val Leu Val Asp Asn Lys Cys
        275                 280                 285

Lys Cys Ala Arg Ile Thr Ser Arg Ile Ile Arg Ser Ser Glu Asp Pro
    290                 295                 300

Asn Glu Asp Ile Val Glu Arg Asn Ile Arg Ile Ile Val Pro Leu Asn
305                 310                 315                 320
```

```
Asn Arg Glu Asn Ile Ser Asp Pro Thr Ser Pro Leu Arg Thr Arg Phe
                325                 330                 335

Val Tyr His Leu Ser Asp Leu Cys Lys Lys Cys Asp Pro Thr Glu Val
            340                 345                 350

Glu Leu Asp Asn Gln Ile Val Thr Ala Thr Gln Ser Asn Ile Cys Asp
        355                 360                 365

Glu Asp Ser Ala Thr Glu Thr Cys Tyr Thr Tyr Asp Arg Asn Lys Cys
    370                 375                 380

Tyr Thr Ala Val Val Pro Leu Val Tyr Gly Gly Glu Thr Lys Met Val
385                 390                 395                 400

Glu Thr Ala Leu Thr Pro Asp Ala Cys Tyr Pro Asp Gly Gly Gly Gly
                405                 410                 415

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ala His Lys Ser
            420                 425                 430

Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala
        435                 440                 445

Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu
    450                 455                 460

Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys
465                 470                 475                 480

Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu
                485                 490                 495

Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly
            500                 505                 510
```

```
Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys
        515                 520                 525

Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg
    530                 535                 540

Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr
545                 550                 555                 560

Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe
                565                 570                 575

Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe
            580                 585                 590

Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys
        595                 600                 605

Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg
    610                 615                 620

Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala
625                 630                 635                 640

Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala
                645                 650                 655

Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys
            660                 665                 670

Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala
        675                 680                 685

Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu
    690                 695                 700

Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val
705                 710                 715                 720
```

```
Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe
               725                 730                 735

Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val
            740                 745                 750

Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr
        755                 760                 765

Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu
    770                 775                 780

Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val
785                 790                 795                 800

Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys
               805                 810                 815

Gln Asn Cys Glu Leu Phe Lys Gln Leu Gly Glu Tyr Lys Phe Gln Asn
            820                 825                 830

Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro
        835                 840                 845

Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys
    850                 855                 860

Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu
865                 870                 875                 880

Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val
               885                 890                 895

Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg
            900                 905                 910
```

```
Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu
        915                 920                 925


Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser
    930                 935                 940


Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val
945                 950                 955                 960


Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp
                965                 970                 975


Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu
            980                 985                 990


Thr Cys Phe Ala Glu Glu Gly Lys  Lys Leu Val Ala Ala  Ser Gln Ala
        995                 1000                1005


Ala Leu  Gly Leu
    1010


<210> 35
<211> 1012
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 35
Met Gly Trp Ser Tyr Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1               5                   10                  15


Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
            20                  25                  30


Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
        35                  40                  45
```

```
Ile Ser Tyr Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
    50                  55                  60

Glu Trp Met Gly Tyr Ile Asn Pro Arg Ser Gly Tyr Thr His Tyr Asn
65                  70                  75                  80

Gln Lys Leu Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ala Ser
                85                  90                  95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Arg Ser Ala Tyr Tyr Asp Tyr Asp Gly Phe Ala Tyr
        115                 120                 125

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser
    130                 135                 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln
145                 150                 155                 160

Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr
                165                 170                 175

Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys
            180                 185                 190

Pro Gly Lys Ala Pro Lys Arg Leu Ile Tyr Asp Thr Ser Lys Leu Ala
        195                 200                 205

Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
    210                 215                 220

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
225                 230                 235                 240

Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys
                245                 250                 255
```

```
Val Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
            260                 265                 270

Gly Gly Ser Gln Glu Asp Glu Arg Ile Val Leu Val Asp Asn Lys Cys
        275                 280                 285

Lys Cys Ala Arg Ile Thr Ser Arg Ile Ile Arg Ser Ser Glu Asp Pro
    290                 295                 300

Asn Glu Asp Ile Val Glu Arg Asn Ile Arg Ile Ile Val Pro Leu Asn
305                 310                 315                 320

Asn Arg Glu Asn Ile Ser Asp Pro Thr Ser Pro Leu Arg Thr Arg Phe
                325                 330                 335

Val Tyr His Leu Ser Asp Leu Cys Lys Lys Cys Asp Pro Thr Glu Val
            340                 345                 350

Glu Leu Asp Asn Gln Ile Val Thr Ala Thr Gln Ser Asn Ile Cys Asp
        355                 360                 365

Glu Asp Ser Ala Thr Glu Thr Cys Ala Thr Tyr Asp Arg Asn Lys Cys
    370                 375                 380

Tyr Thr Ala Val Val Pro Leu Val Tyr Gly Gly Glu Thr Lys Met Val
385                 390                 395                 400

Glu Thr Ala Leu Thr Pro Asp Ala Cys Tyr Pro Asp Gly Gly Gly Gly
                405                 410                 415

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ala His Lys Ser
            420                 425                 430

Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala
        435                 440                 445
```

```
Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu
    450                 455                 460

Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys
465                 470                 475                 480

Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu
                485                 490                 495

Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly
            500                 505                 510

Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys
        515                 520                 525

Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg
    530                 535                 540

Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr
545                 550                 555                 560

Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe
                565                 570                 575

Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe
            580                 585                 590

Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys
        595                 600                 605

Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg
    610                 615                 620

Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala
625                 630                 635                 640

Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala
                645                 650                 655
```

```
Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys
            660                 665                 670

Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala
        675                 680                 685

Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu
    690                 695                 700

Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val
705                 710                 715                 720

Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe
                725                 730                 735

Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val
            740                 745                 750

Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr
        755                 760                 765

Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu
    770                 775                 780

Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val
785                 790                 795                 800

Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys
                805                 810                 815

Gln Asn Cys Glu Leu Phe Lys Gln Leu Gly Glu Tyr Lys Phe Gln Asn
            820                 825                 830

Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro
        835                 840                 845
```

```
Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys
    850                 855                 860

Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu
865                 870                 875                 880

Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val
                885                 890                 895

Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg
            900                 905                 910

Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu
        915                 920                 925

Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser
    930                 935                 940

Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val
945                 950                 955                 960

Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp
                965                 970                 975

Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu
            980                 985                 990

Thr Cys Phe Ala Glu Glu Gly Lys  Lys Leu Val Ala Ala  Ser Gln Ala
        995                 1000                1005

Ala Leu  Gly Leu
    1010
```

<210> 36
<211> 393
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 36

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ile Ser Tyr
            20                  25                  30


Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Tyr Ile Asn Pro Arg Ser Gly Tyr Thr His Tyr Asn Gln Lys Leu
    50                  55                  60


Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ala Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Ser Ala Tyr Tyr Asp Tyr Asp Gly Phe Ala Tyr Trp Gly Gln
            100                 105                 110


Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
        115                 120                 125


Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser
    130                 135                 140


Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala
145                 150                 155                 160


Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys
                165                 170                 175


Ala Pro Lys Arg Leu Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val
            180                 185                 190
```

```
Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
        195                 200                 205

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
    210                 215                 220

Trp Ser Ser Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
225                 230                 235                 240

Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
                245                 250                 255

Gln Glu Asp Glu Arg Ile Val Leu Val Asp Asn Lys Cys Lys Cys Ala
            260                 265                 270

Arg Ile Thr Ser Arg Ile Ile Arg Ser Ser Glu Asp Pro Asn Glu Asp
        275                 280                 285

Ile Val Glu Arg Asn Ile Arg Ile Ile Val Pro Leu Asn Asn Arg Glu
    290                 295                 300

Asn Ile Ser Asp Pro Thr Ser Pro Leu Arg Thr Arg Phe Val Tyr His
305                 310                 315                 320

Leu Ser Asp Leu Cys Lys Lys Cys Asp Pro Thr Glu Val Glu Leu Asp
                325                 330                 335

Asn Gln Ile Val Thr Ala Thr Gln Ser Asn Ile Cys Asp Glu Asp Ser
            340                 345                 350

Ala Thr Glu Thr Cys Tyr Thr Tyr Asp Arg Asn Lys Cys Tyr Thr Ala
        355                 360                 365

Val Val Pro Leu Val Tyr Gly Gly Glu Thr Lys Met Val Glu Thr Ala
    370                 375                 380
```

Leu Thr Pro Asp Ala Cys Tyr Pro Asp
385 390

<210> 37
<211> 393
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 37
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ile Ser Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Tyr Ile Asn Pro Arg Ser Gly Tyr Thr His Tyr Asn Gln Lys Leu
50 55 60

Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ala Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Ala Tyr Tyr Asp Tyr Asp Gly Phe Ala Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser
130 135 140

```
Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala
145                 150                 155                 160


Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys
                165                 170                 175


Ala Pro Lys Arg Leu Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val
            180                 185                 190


Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
        195                 200                 205


Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
    210                 215                 220


Trp Ser Ser Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
225                 230                 235                 240


Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
                245                 250                 255


Gln Glu Asp Glu Arg Ile Val Leu Val Asp Asn Lys Cys Lys Cys Ala
            260                 265                 270


Arg Ile Thr Ser Arg Ile Ile Arg Ser Ser Glu Asp Pro Asn Glu Asp
        275                 280                 285


Ile Val Glu Arg Asn Ile Arg Ile Ile Val Pro Leu Asn Asn Arg Glu
    290                 295                 300


Asn Ile Ser Asp Pro Thr Ser Pro Leu Arg Thr Arg Phe Val Tyr His
305                 310                 315                 320


Leu Ser Asp Leu Cys Lys Lys Cys Asp Pro Thr Glu Val Glu Leu Asp
                325                 330                 335


Asn Gln Ile Val Thr Ala Thr Gln Ser Asn Ile Cys Asp Glu Asp Ser
            340                 345                 350
```

Ala Thr Glu Thr Cys Ala Thr Tyr Asp Arg Asn Lys Cys Tyr Thr Ala
        355                 360                 365

Val Val Pro Leu Val Tyr Gly Gly Glu Thr Lys Met Val Glu Thr Ala
    370                 375                 380

Leu Thr Pro Asp Ala Cys Tyr Pro Asp
385                 390

<210> 38
<211> 420
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 38
Met Gly Trp Ser Tyr Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1               5                   10                  15

Val His Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
            20                  25                  30

Pro Lys Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
        35                  40                  45

Asn Thr Tyr Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
    50                  55                  60

Glu Trp Val Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr
65                  70                  75                  80

Tyr Ala Asp Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser
                85                  90                  95

Gln Ser Ile Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr
            100                 105                 110

```
Ala Met Tyr Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val
        115                 120                 125

Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
    130                 135                 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln
145                 150                 155                 160

Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr
                165                 170                 175

Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn
            180                 185                 190

Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly Leu
        195                 200                 205

Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser
    210                 215                 220

Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln
225                 230                 235                 240

Thr Glu Asp Glu Ala Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn Leu
                245                 250                 255

Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gly Gly Gly
            260                 265                 270

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Glu Asp Glu Arg
        275                 280                 285

Ile Val Leu Val Asp Asn Lys Cys Lys Cys Ala Arg Ile Thr Ser Arg
    290                 295                 300
```

Ile Ile Arg Ser Ser Glu Asp Pro Asn Glu Asp Ile Val Glu Arg Asn
305 310 315 320

Ile Arg Ile Ile Val Pro Leu Asn Asn Arg Glu Asn Ile Ser Asp Pro
325 330 335

Thr Ser Pro Leu Arg Thr Arg Phe Val Tyr His Leu Ser Asp Leu Cys
340 345 350

Lys Lys Cys Asp Pro Thr Glu Val Glu Leu Asp Asn Gln Ile Val Thr
355 360 365

Ala Thr Gln Ser Asn Ile Cys Asp Glu Asp Ser Ala Thr Glu Thr Cys
370 375 380

Ala Thr Tyr Asp Arg Asn Lys Cys Tyr Thr Ala Val Val Pro Leu Val
385 390 395 400

Tyr Gly Gly Glu Thr Lys Met Val Glu Thr Ala Leu Thr Pro Asp Ala
405 410 415

Cys Tyr Pro Asp
420

<210> 39
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic primer

<400> 39
gaccgacgtg tgctactatt ac 22

<210> 40
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 40
gaggtgcttc acgagattct t 21

<210> 41
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 41
Gly Gly Gly Gly Ser
1 5

<210> 42
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 42
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10

<210> 43
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 43
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 44
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 44
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser
20

<210> 45
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 45
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25

<210> 46
<211> 455
<212> PRT
<213> Mus musculus

<400> 46
Ala Ser Gln Ser Phe Pro Asn Val Phe Pro Leu Val Ser Cys Glu Ser
1 5 10 15

Pro Leu Ser Asp Lys Asn Leu Val Ala Met Gly Cys Leu Ala Arg Asp
20 25 30

```
Phe Leu Pro Ser Thr Ile Ser Phe Thr Trp Asn Tyr Gln Asn Asn Thr
        35                  40                  45

Glu Val Ile Gln Gly Ile Arg Thr Phe Pro Thr Leu Arg Thr Gly Gly
    50                  55                  60

Lys Tyr Leu Ala Thr Ser Gln Val Leu Leu Ser Pro Lys Ser Ile Leu
65                  70                  75                  80

Glu Gly Ser Asp Glu Tyr Leu Val Cys Lys Ile His Tyr Gly Gly Lys
                85                  90                  95

Asn Arg Asp Leu His Val Pro Ile Pro Ala Val Ala Glu Met Asn Pro
            100                 105                 110

Asn Val Asn Val Phe Val Pro Pro Arg Asp Gly Phe Ser Gly Pro Ala
        115                 120                 125

Pro Arg Lys Ser Lys Leu Ile Cys Glu Ala Thr Asn Phe Thr Pro Lys
    130                 135                 140

Pro Ile Thr Val Ser Trp Leu Lys Asp Gly Lys Leu Val Glu Ser Gly
145                 150                 155                 160

Phe Thr Thr Asp Pro Val Thr Ile Glu Asn Lys Gly Ser Thr Pro Gln
                165                 170                 175

Thr Tyr Lys Val Ile Ser Thr Leu Thr Ile Ser Glu Ile Asp Trp Leu
            180                 185                 190

Asn Leu Asn Val Tyr Thr Cys Arg Val Asp His Arg Gly Leu Thr Phe
        195                 200                 205

Leu Lys Asn Val Ser Ser Thr Cys Ala Ala Ser Pro Ser Thr Asp Ile
    210                 215                 220

Leu Asn Phe Thr Ile Pro Pro Ser Phe Ala Asp Ile Phe Leu Ser Lys
225                 230                 235                 240
```

```
Ser Ala Asn Leu Thr Cys Leu Val Ser Asn Leu Ala Thr Tyr Glu Thr
                245                 250                 255

Leu Ser Ile Ser Trp Ala Ser Gln Ser Gly Glu Pro Leu Glu Thr Lys
            260                 265                 270

Ile Lys Ile Met Glu Ser His Pro Asn Gly Thr Phe Ser Ala Lys Gly
        275                 280                 285

Val Ala Ser Val Cys Val Glu Asp Trp Asn Asn Arg Lys Glu Phe Val
    290                 295                 300

Cys Thr Val Thr His Arg Asp Leu Pro Ser Pro Gln Lys Lys Phe Ile
305                 310                 315                 320

Ser Lys Pro Asn Glu Val His Lys His Pro Pro Ala Val Tyr Leu Leu
                325                 330                 335

Pro Pro Ala Arg Glu Gln Leu Asn Leu Arg Glu Ser Ala Thr Val Thr
            340                 345                 350

Cys Leu Val Lys Gly Phe Ser Pro Ala Asp Ile Ser Val Gln Trp Lys
        355                 360                 365

Gln Arg Gly Gln Leu Leu Pro Gln Glu Lys Tyr Val Thr Ser Ala Pro
    370                 375                 380

Met Pro Glu Pro Gly Ala Pro Gly Phe Tyr Phe Thr His Ser Ile Leu
385                 390                 395                 400

Thr Val Thr Glu Glu Glu Trp Asn Ser Gly Glu Thr Tyr Thr Cys Val
                405                 410                 415

Val Gly His Glu Ala Leu Pro His Leu Val Thr Glu Arg Thr Val Asp
            420                 425                 430
```

```
Lys Ser Thr Gly Lys Pro Thr Leu Tyr Asn Val Ser Leu Ile Met Ser
        435                 440                 445


Asp Thr Gly Gly Thr Cys Tyr
    450                 455


<210> 47
<211> 474
<212> PRT
<213> Macaca fascicularis

<400> 47
Glu Ser Ala Gly Pro Phe Lys Trp Glu Pro Ser Val Ser Ser Pro Asn
1               5                   10                  15


Ala Pro Leu Asp Thr Asn Glu Val Ala Val Gly Cys Leu Ala Gln Asp
            20                  25                  30


Phe Leu Pro Asp Ser Ile Thr Phe Ser Trp Lys Phe Lys Asn Asn Ser
        35                  40                  45


Asp Ile Ser Lys Gly Val Trp Gly Phe Pro Ser Val Leu Arg Gly Gly
    50                  55                  60


Lys Tyr Ala Ala Thr Ser Gln Val Leu Leu Ala Ser Lys Asp Val Met
65                  70                  75                  80


Gln Gly Thr Asp Glu His Val Val Cys Lys Val Gln His Pro Asn Gly
                85                  90                  95


Asn Lys Glu Gln Asn Val Pro Leu Pro Val Val Ala Glu Arg Pro Pro
            100                 105                 110


Asn Val Ser Val Phe Val Pro Pro Arg Asp Gly Phe Val Gly Asn Pro
        115                 120                 125


Arg Glu Ser Lys Leu Ile Cys Gln Ala Thr Gly Phe Ser Pro Arg Gln
    130                 135                 140
```

```
Ile Glu Val Ser Trp Leu Arg Asp Gly Lys Gln Val Gly Ser Gly Ile
145                 150                 155                 160

Thr Thr Asp Arg Val Glu Ala Glu Ala Lys Glu Ser Gly Pro Thr Thr
                165                 170                 175

Phe Lys Val Thr Ser Thr Leu Thr Val Ser Glu Arg Asp Trp Leu Ser
            180                 185                 190

Gln Ser Val Phe Thr Cys Arg Val Asp His Arg Gly Leu Thr Phe Gln
        195                 200                 205

Lys Asn Val Ser Ser Val Cys Gly Pro Asn Pro Asp Thr Ala Ile Arg
    210                 215                 220

Val Phe Ala Ile Pro Pro Ser Phe Ala Ser Ile Phe Leu Thr Lys Ser
225                 230                 235                 240

Thr Lys Leu Thr Cys Leu Val Thr Asp Leu Ala Thr Tyr Asp Ser Val
                245                 250                 255

Thr Ile Thr Trp Thr Arg Gln Asn Gly Glu Ala Leu Lys Thr His Thr
            260                 265                 270

Asn Ile Ser Glu Ser His Pro Asn Gly Thr Phe Ser Ala Val Gly Glu
        275                 280                 285

Ala Ser Ile Cys Glu Asp Asp Trp Asn Ser Gly Glu Arg Phe Arg Cys
    290                 295                 300

Thr Val Thr His Thr Asp Leu Pro Ser Pro Leu Lys Gln Thr Ile Ser
305                 310                 315                 320

Arg Pro Lys Gly Val Ala Met His Arg Pro Asp Val Tyr Leu Leu Pro
                325                 330                 335

Pro Ala Arg Glu Gln Leu Asn Leu Arg Glu Ser Ala Thr Ile Thr Cys
            340                 345                 350
```

```
Leu Val Thr Gly Phe Ser Pro Ala Asp Ile Phe Val Gln Trp Met Gln
        355                 360                 365


Arg Gly Gln Pro Leu Ser Pro Glu Lys Tyr Val Thr Ser Ala Pro Met
    370                 375                 380


Pro Glu Pro Gln Ala Pro Gly Arg Tyr Phe Ala His Ser Ile Leu Thr
385                 390                 395                 400


Val Ser Glu Glu Asp Trp Asn Thr Gly Glu Thr Tyr Thr Cys Val Val
                405                 410                 415


Ala His Glu Ala Leu Pro Asn Arg Val Thr Glu Arg Thr Val Asp Lys
            420                 425                 430


Ser Thr Gly Lys Pro Thr Leu Tyr Asn Val Ser Leu Val Ile Leu Trp
        435                 440                 445


Thr Thr Leu Ser Thr Phe Val Ala Leu Phe Val Leu Thr Leu Leu Tyr
    450                 455                 460


Ser Gly Ile Val Thr Phe Ile Lys Val Arg
465                 470


<210> 48
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide


<220>
<221> MISC_FEATURE
<222> (1)..(25)
<223> This sequence may encompass 1-5 "Gly Gly Gly Gly Ser"
      repeating units
```

<220>
<223> See specification as filed for detailed description of
substitutions and preferred embodiments

<400> 48
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25