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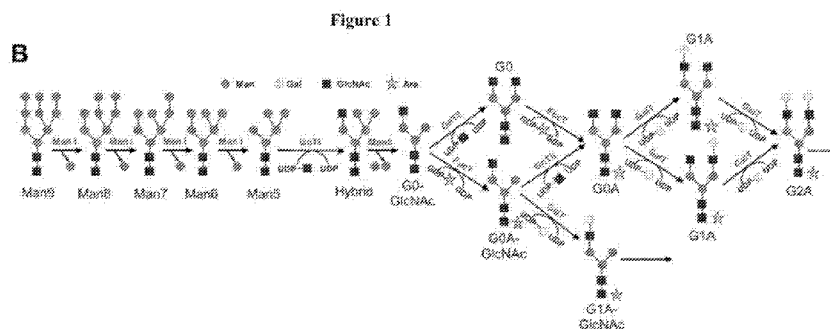
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(57) Abstract: The present invention relates to the field of protein production and, in particular, to methods and compositions for modulating glycosylation of proteins expressed by host cells. In particular glycoproteins are produced in presence of arabinose and/or altrose, whereby N-linked glycans are afucosylated and replaced with arabinosylated N-linked glycans.

ARABINOSYLATED GLYCOPROTEINS

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/129,458, filed on 6 March 2015; the entire contents of said application are incorporated herein in their entirety by this reference.

FIELD OF THE INVENTION

[0002] The present invention relates to therapeutic glycoproteins and, in particular, arabinosylated glycoproteins. The present invention also relates to methods for making and using arabinosylated glycoproteins. The present invention also relates to methods for making and using glycoprotein compositions having a reduced amount of core fucose residues and/or a reduced amount of high mannose residues.

BACKGROUND OF THE INVENTION

[0003] There are two fundamental types of protein glycosylation corresponding to the amino acid with which the oligosaccharides are added onto the protein: Asn-linked (N-linked) glycosylation and Ser/Thr-linked (O-linked) glycosylation. N-linked glycosylation is commonly found in recombinant glycoprotein therapeutics, and follows a reasonably understood series of enzymatic events (Hossler et al., *Biotechnol. Bioeng.* 95:946-960 (2006)). The pathway begins in the endoplasmic reticulum (ER) where there is an en bloc transfer of a 9-mannose core N-glycan oligosaccharide. Following a series of mannose trimming reactions, there is a step-wise series of additions of various monosaccharides onto the N-glycan in the Golgi apparatus. The addition of these monosaccharides follows a set of enzymatic reactions that are determined by a variety of factors including enzymatic substrate specificity. The donor substrates for these monosaccharide addition reactions are nucleotide sugars which are recognized by glycosyltransferase enzymes for subsequent addition onto the N-glycan. Sugar-by-sugar the N-glycan is then built up along the protein secretory pathway, before eventually being transferred over to the protein's final destination. In the case of most recombinant glycoprotein therapeutics expressed from mammalian cells, this is essentially constitutive secretion into the extracellular environment. There are only a handful of different glycosidase enzymes, glycosyltransferase enzymes, and nucleotide-

sugars in the N-glycan biosynthetic pathway. However, due to a variety of reasons, there exists a great deal of variability in this metabolic pathway, which gives rise to a diverse spectrum of different N-glycans on the expressed protein. This spectrum of N-glycans is termed the glycoform profile. Differences in the monosaccharide composition that comprise the N-glycans are termed microheterogeneity. Differences in the N-glycan occupancy across the respective glycosylation sites of different protein molecules are termed macroheterogeneity.

[0004] Control over N-glycan content is important for the commercial manufacturing of recombinant glycoprotein therapeutics for optimization of efficacy, ensuring consistency between repeated batches of the protein product, and directing product quality profiles (e.g., directing a product profile towards a comparability fingerprint as that of a desired reference material).

[0005] Mannose and fucose are two types of sugars attached to N-glycans on recombinant proteins. A pre-formed dolichol oligosaccharide unit is first added onto a glycoprotein upon translation in the lumen of the ER. Glucose and mannose are serially removed from N-glycans while in the ER. Frequently, mannose removal is not complete before the protein exits the ER and eventually makes its way outside the cell through the secretory pathway. Mannose is an important sugar that has been implicated in regulating cellular activities in a variety of studies (Sathyamoorthy et al., *Mol. Cell Biochem.* 102:139-147 (1991)). For example, high mannose N-glycans can resemble the glycosylation profile from proteins of adventitious organisms (Allavena et al., *Crit. Rev. Immunol.* 24:179-192 (2004)), and thus potentially induce both an immunogenic response, as well as a reduced pharmacokinetic profile (PK) (Yu, et al., *mAbs* 4:475-487 (2012); Goetze et al., *Glycobiol.* 21:949-959 (2011)). The mannose receptor is a receptor principally found on dendritic cells and macrophages and has been found to be principally important towards the recognition of high mannose epitopes and the resulting clearance of the proteins which display them (Allavena et al., *Crit. Rev. Immunol.* 24:179-192 (2004); Stahl, *Curr. Opin. Immunol.* 4:49-52 (1992); Lee et al., *Science* 295:1898-1901 (2002)).

[0006] Fucose is one of the rare sugars that is actually added onto an N-glycan as the L- form of the sugar. Fucose has been implicated in various reports suggesting that lower levels of L-fucose attached to an N-glycan can increase antibody-dependent cell cytotoxicity (ADCC) (Shields et al., *J. Biol. Chem.* 277:26733 (2002); Shinkawa et al., *J. Biol. Chem.* 278:3466-3473 (2003)) and that this effect is dependent upon activity of

natural killer cells (Peipp et al., Blood 112:2390-2399 (2008)). This latter effect is of contemporary interest in oncology-related protein therapeutics in particular, where the ADCC response is typically the molecule's principal method of action (Chan and Carter, Nat. Rev. Immunol. 10:301-316 (2010)).

[0007] The mannose and fucose content of a therapeutic glycoprotein composition can impact the efficacy and/or PK profile of the therapeutically relevant glycoproteins. For most recombinant glycoprotein therapeutics it is desirable to have a sufficiently long PK associated with a relatively low risk for immunogenicity or inflammatory response. For others, especially oncology-related therapeutics, it is desirable to have a high ADCC activity to affect greater tumor cell killing. Since the relative levels of mannose and fucose have been shown to play an important role towards these physiological responses of the proteins which bear them, having a means with which to control their relative levels would be extremely beneficial for the targeted optimization of therapeutic safety and efficacy. This is especially important for the commercial manufacturing of recombinant glycoprotein therapeutics for both the targeted optimization of efficacy, as well as ensuring consistency between repeated batches of the protein product, and directing product quality profiles towards a comparability fingerprint as that of a desired reference material. Galactose is an interesting sugar that is added during the later stages of glycan processing, towards the end of N-glycans and is frequently considered part of terminal glycosylation. *In vitro* studies have shown how antibodies that are deficient in galactose content have an enhanced binding activity to the mannose binding lectin (MBL) (Malhotra et al., Nat. Med. 1:237-243 (1995)). *In vivo* studies have shown how antibodies that are deficient in galactose content have a fixed conformation that more heavily favors binding to FcR's, and thus cellular activation of effector cells, including those associated with inflammatory responses (Nimmerjahn et al., Proc. Natl. Acad. Sci. USA 104:8433-8437)).

[0008] Accordingly, an ideal oligosaccharide profile for many glycoprotein therapeutics, whether it be for oncology or immunology indications, is one that is high in galactose, low in fucose, and low in mannose. However, it is not easy to control the levels of one of these particular sugars at a time, let alone control all three variables at the same time without introducing any adverse changes to the expression system's cell growth performance, recombinant protein productivity, and/or other product quality attributes. The difficulty in modulating protein glycosylation patterns of commercial and pre-clinical glycoprotein therapeutics is further evidenced by the divergent technologies applied to

modify such glycosylation patterns (Beck and Reichert, mAbs 4:419-425 (2012), especially at Tables 1 and 2). While reports highlight the degree to which a glycoprotein's final N-glycan profile is mediated through a number of variables, such as the cell line being used to express the protein, the protein itself, the process used to culture the cells, as well as the various parameters used to culture the cells, individual compounds in the culture media of the cells used to express the protein can also significantly determine the glycoprotein's final N-glycan profile (Hossler et al., Glycobiol. 19:936-949 (2009)). Accordingly, there is a great need in the art to identify engineered glycoproteins and methods of making and using same having modified desirable glycosylation profiles particularly wherein individual culture media components can be modulated in order to modulate the glycoprotein's N-glycan profile.

BRIEF SUMMARY OF THE INVENTION

[0009] The various aspects of the present invention are based, at least in part, on the discoveries described herein related to compositions and methods for regulating the levels of high mannose N-glycans and N-glycan fucose of glycoproteins, such as antibodies like monoclonal antibodies (mAbs) and dual variable domain proteins (DVDs), through the cell culture media supplementation of D-arabinose and/or D-altrose during the production of the glycoproteins. In particular, D-arabinose and D-altrose were able to significantly and selectively reduce fucosylation within N-glycans (*e.g.*, completely replace fucose within core antibody N-glycans) across multiple glycoproteins, as well as significantly reduce mannosylation, reduce dendritic cell uptake, increase FcγRIIIa binding, maintain (preserve) or enhance complement activation, enhance antibody-dependent cell-mediated cytotoxicity (ADCC), reduce immunogenicity, and increase circulatory pharmacokinetics (PK), of the glycoproteins. Moreover, such effects were obtained without appreciable adverse modulation of inflammatory cytokine release signature, complement activation, or *in vivo* PK.

[0010] These and other objects of the invention are described in the following paragraphs. These objects should not be deemed to narrow the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] **Figure 1** includes 2 panels, identified as panels (A) and (B), which depict an abbreviated N-glycan biosynthetic pathway in mammalian cells. Panel A shows an

abbreviated N-glycan biosynthetic pathway in mammalian cells using L-fucose as a sugar substrate. Panel B shows an abbreviated N-glycan biosynthetic pathway in mammalian cells using D-arabinose as a sugar substrate.

[0012] **Figure 2** depicts the chemical structure comparison between D-arabinose, D-altrose, and L-fucose, highlighting the similarity in structure and stereochemistry at select carbon positions.

[0013] **Figure 3** includes 3 panels, identified as panels (A), (B), and (C), which depict cell culture performance of a mAb-1-expressing CHO cell line with different concentrations of D-arabinose supplemented into the culture media. Panel A shows results regarding viable cell density. Panel B shows results regarding cell viability. Panel C shows results regarding relative harvest titer.

[0014] **Figure 4** includes 2 panels, identified as panels (A) and (B), which depict a reduced LC-MS spectragraph of purified mAb-1 expressed in CHO cell culture under different conditions. Panel A shows the results of a control culture with glucose as the principal sugar. Panel B shows the results of an experimental culture with glucose and D-arabinose as the principal sugars.

[0015] **Figure 5** includes 2 panels, identified as panels (A) and (B), which depict a Lys-C treated, and reduced LC-MS spectragraph of a purified mAb-1 expressed in CHO cell culture with different sugar supplements. Panel A shows the results of D-arabinose. Panel B shows the results of ¹³C labeled D-arabinose.

[0016] **Figure 6** shows the results of N-glycan glycopeptide mapping of purified mAb-1 expressed from CHO cells supplemented with D-altrose or D-arabinose.

[0017] **Figure 7** includes 3 panels, identified as panels (A), (B), and (C), which depict cell culture performance of an adalimumab-expressing CHO cell line with either sucrose or D-arabinose supplemented into the culture media. Panel A shows results regarding viable cell density. Panel B shows results regarding cell viability. Panel C shows results regarding relative harvest titer.

[0018] **Figure 8** includes 3 panels, identified as panels (A), (B), and (C), which depict cell culture performance of a DVD-1-expressing CHO cell line with different concentrations of D-arabinose supplemented into the culture media. Panel A shows results regarding viable cell density. Panel B shows results regarding cell viability. Panel C shows results regarding relative harvest titer.

[0019] **Figure 9** includes 3 panels, identified as panels (A), (B), and (C), which depict cell culture performance of a mAb-2-expressing CHO cell line with different concentrations of D-arabinose supplemented into the culture media. Panel A shows results regarding viable cell density. Panel B shows results regarding cell viability. Panel C shows results regarding relative harvest titer.

[0020] **Figure 10** includes 3 panels, identified as panels (A), (B), and (C), which depict cell culture performance of a mAb-3-expressing CHO cell line with different concentrations of D-arabinose supplemented into the culture media. Panel A shows results regarding viable cell density. Panel B shows results regarding cell viability. Panel C shows results regarding relative harvest titer.

[0021] **Figure 11** depicts dendritic cell uptake data of a purified anti-TNF α antibody expressed in CHO cell culture with different sugar supplements.

[0022] **Figure 12** includes 2 panels, identified as panels (A) and (B), which depict complement activation elicited by purified mAb-1 expressed in CHO cell culture with different sugar supplements. Panel A shows the response from cells from human donor 1. Panel B shows the response from cells from human donor 2.

[0023] **Figure 13** includes 2 panels, identified as panels (A) and (B), which depict rat PK results of glycomodulated mAb-1 mediated through D-arabinose cell culture media supplementation. Panel A shows the results of intravenous administration. Panel B shows the results of subcutaneous administration.

[0024] **Figure 14** includes 2 panels, identified as panels (A) and (B), which depict ADCC measurements of arabinosylated mAb-2 versus predominantly fucosylated control mAb-2. Panel A shows the results of the V158 higher affinity allele of FC γ RIIIa. Panel B shows the results of the F158 lower affinity allele of FC γ RIIIa.

[0025] **Figure 15** includes 2 panels, identified as panels (A) and (B), which depict CD16 report assay results of predominantly fucosylated control mAb-3 versus predominantly arabinosylated mAb-3. Panel A shows the results of the V158 higher affinity allele of FC γ RIIIa. Panel B shows the results of the F158 lower affinity allele of FC γ RIIIa.

[0026] **Figure 16** includes 3 panels, identified as panels (A), (B), and (C), which depict cell culture performance of a mAb-1-expressing CHO cell line with different concentrations of D-arabinose and manganese supplemented into the culture media. Panel A shows results regarding viable cell density. Panel B shows results regarding cell viability. Panel C shows results regarding relative harvest titer.

DETAILED DESCRIPTION OF THE INVENTION

[0027] This detailed description is intended to acquaint others skilled in the art with the present invention, its principles, and its practical application so that others skilled in the art may adapt and apply the invention in its numerous forms, as they may be best suited to the requirements of a particular use. This description and its specific examples are intended for purposes of illustration. This invention, therefore, is not limited to the embodiments described in this patent application, and may be variously modified.

[0028] The present invention provides a recombinant glycoprotein, such as a therapeutic protein (e.g., antibody, DVD-Ig, TVD-Ig, Half-body or receptor antibody (“RAB”) compositions) having a modulated glycosylation profile. In certain embodiments, the modulated glycosylation profile includes increased arabinosylation, decreased fucosylation, and/or decreased mannosylation relative to a control protein.

[0029] The present invention further provides a pharmaceutical composition comprising a recombinant glycoprotein, such as a therapeutic protein, having a modulated glycosylation profile. In certain embodiments, the modulated glycosylation profile includes increased arabinosylation, decreased fucosylation, and/or decreased mannosylation relative to a control protein. In certain embodiments, the therapeutic protein is an antibody.

[0030] The present invention provides cell culture methods and compositions for modulating the glycosylation profile of a protein such as a therapeutic protein. In certain embodiments, the cell culture methods comprise culturing a host cell expressing the protein in cell culture media supplemented with D-arabinose and/or D-altrose. In certain embodiments, the cell culture composition comprises D-arabinose and/or D-altrose.

[0031] A. DEFINITIONS

[0032] As used in the specification and the appended claims, unless specified to the contrary, the following terms have the meaning indicated:

[0033] The articles “a” and “an” are used herein to refer to one or two more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an arabinose residue” means one arabinose residue or more than one arabinose residue.

[0034] The term “antibody” refers to an immunoglobulin molecule and immunologically active portions of immunoglobulin molecules, i.e., polypeptides of the immunoglobulin family, or fragments thereof, that contain an antigen binding site that immunospecifically binds to a specific antigen (e.g., TNF α) and an Fc domain. The term

“antibody” includes an immunoglobulin molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as “HCVR” or “VH”) and a heavy chain constant region (“CH”). The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as “LCVR” or “VL”) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (“CDRs”), interspersed with regions that are more conserved, termed framework regions (“FR”). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[0035] The term “antigen-binding portion” or “antigen-binding fragment” of an antibody includes fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., TNF α). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of antigen-binding fragments encompassed within the terms “antigen-binding portion” or “antigen-binding fragment” of an antibody include (i) a Fab fragment, a monovalent fragment comprising the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment comprising the VH and CH1 domains; (iv) a Fv fragment comprising the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546, the entire teaching of which is incorporated herein by reference), which comprises a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see, e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883, the entire teachings of which are incorporated herein by reference). Such single chain antibodies are also intended to be encompassed within the terms “antigen-binding portion” or “antigen-binding fragment” of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed by the terms “antigen-binding portion” or “antigen-

binding fragment” of an antibody. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see, e.g., Holliger, P., et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R. J., et al. (1994) *Structure* 2:1121-1123, the entire teachings of which are incorporated herein by reference).

[0036] In certain embodiments, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecule, formed by covalent or non-covalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S. M., et al. (1995) *Human Antibodies and Hybridomas* 6:93-101, the entire teaching of which is incorporated herein by reference) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov, S. M., et al. (1994) *Mol. Immunol.* 31:1047-1058, the entire teaching of which is incorporated herein by reference).

[0037] The term “monoclonal antibody” refers to an antibody produced by a single clone of B-lymphocytes or by a cell into which the light and heavy chain genes of a single antibody have been transfected. Monoclonal antibodies are produced by methods known to those of skill in the art, for instance by making hybrid antibody-forming cells from a fusion of myeloma cells with immune spleen cells. Monoclonal antibodies include murine, human, humanized and chimeric monoclonal antibodies.

[0038] The term “isolated antibody” includes an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds hTNF α is substantially free of antibodies that specifically bind antigens other than hTNF α). An isolated antibody that specifically binds hTNF α may bind TNF α molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals. A suitable anti-TNF α antibody is adalimumab or an adalimumab-like polypeptide.

[0039] As used herein, the term “adalimumab,” also known by its trade name HUMIRA® (AbbVie) and sometimes referred to as “D2E7”, refers to a human IgG1 antibody that binds human tumor necrosis factor α (TNF α). The light chain variable region of adalimumab and the heavy chain variable region of adalimumab are provided herein as

shown in Table A. Adalimumab comprises a light chain variable region comprising a CDR1, a CDR2, and a CDR3 as shown in Table A. Adalimumab comprises a heavy chain variable region comprising a CDR1, a CDR2, and a CDR3 of SEQ ID NO:4. The nucleic acid sequence of the light chain variable region and the nucleic acid sequence of the heavy chain variable region are each also set forth in Table A. The full length amino acid sequence of the light chain and the full length amino acid sequence of the heavy chain are also set forth in Table A. In general, the heavy chain constant domain 2 ("CH2") of the adalimumab IgG-Fc region is glycosylated through covalent attachment of oligosaccharide at asparagine 297 (Asn-297). Adalimumab is described in U.S. Pat. Nos. 6,090,382; 6,258,562; 6,509,015; 7,223,394; 7,541,031; 7,588,761; 7,863,426; 7,919,264; 8,197,813; 8,206,714; 8,216,583; 8,420,081; 8,092,998; 8,093,045; 8,187,836; 8,372,400; 8,034,906; 8,436,149; 8,231,876; 8,414,894; 8,372,401, the entire contents of each which are expressly incorporated herein by reference in their entireties. Adalimumab is also described in the "Highlights of Prescribing Information" for HUMIRA® (adalimumab) Injection (Revised January 2008) the contents of which are hereby incorporated herein by reference.

[0040] Table A

D2E7 (Light Chain)

DIQMTQSPSSLSASVGDRVTITC**RASQGIRNYLA**WYQQKPGKAPKLLIY**AASTLQS**
 GVPSRFGSGSGTDFTLTISSLQPEDVATYYC**QRYNRPYTF**GGGTKVEIKRTVAAP
SVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

D2E7 (Heavy Chain)

EVQLVESGGGLVQPGRSLRLSCAASGFTFD**DYAMH**WVRQAPGKGLEWV**SAITWN**
SGHIDYADSVEGRFTISRDNKNSLYLQMNSLRAEDTAVYYCAK**VSYLSTASSLD**
YWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSFSVMHEALHNHYTQKSLSLSPGK

Key

CDR sequences: Bold underlined black text

[CDR1, CDR2, and CDR3 are shown in consequence order from N-terminus to C-terminus (e.g., RASQGIRNYLA represents the VL CDR1 sequence)]

Variable region sequences: Regular text

Constant region sequences: Italicized text

* In some embodiments, the CDR H1 is GFTFDDYAMH instead of DYAMH due to an alternative and well-known antibody CDR nomenclature system. Either CDR H1 sequence annotation can be applied according to the present invention.

[0041] As used herein, the term “adalimumab-like protein” refers to a polypeptide having less than 100% identity, but at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or any range in between, identity to the polypeptide sequence of adalimumab. In one embodiment, the percentage identities refer to the light chain variable region of adalimumab and/or the heavy chain variable region of adalimumab. In another embodiment, the percentage identities refer to CDR1, CDR2, and/or CDR3 of the light chain variable region of adalimumab and/or the CDR1, CDR2, and/or CDR3 of the heavy chain variable region of adalimumab. In still another embodiment, the percentage identities refer to CDR3 of the light chain variable region of adalimumab and/or the CDR3 of the heavy chain variable region of adalimumab. Non-limiting representative examples of adalimumab-like proteins are shown below in Table B.

[0042] Table B

Deimmunized D2E7 sequences with traditional CH region

D2E7-SS (Light Chain)

DIQMTQSPSSLSASVGDRVTITCRASQSIRNYLSWYQQKPGKAPKLLIYAAASTLOS
GVPSRFSGSGSGTDFTLTISLQPEDVATYYCQRYNRPYTFGQGTKVEIKRTVAAP
SVFIFPPSDEQLKSGTASVCLLNFPYFREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

D2E7-SS (Heavy Chain)

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWN
SGHIDYADSVEGFRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLD
YWGQGLTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

Deimmunized D2E7 sequences with mutated CH region

D2E7-SS-QL (Light Chain)

DIQMTQSPSSLSASVGDRVTITCRASQSIRNYLSWYQQKPGKAPKLLIYAAASTLOS
GVPSRFSGSGSGTDFTLTISLQPEDVATYYCQRYNRPYTFGQGTKVEIKRTVAAP
SVFIFPPSDEQLKSGTASVCLLNFPYFREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

D2E7-SS-QL (Heavy Chain)

EVQLVESGGGLVQPGRSLRLSCAASGFTFD**DYAMH**WVRQAPGKGLEWVS**AITWN**
SGHIDYADSVEGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAK**VS****SYLSTASSLD**
YWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
 CPPCPAPELLGGPSVFLFPPKPKD**Q**LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
 VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
 GSFFLYSKLTVDKSRWQQGNVFSCSV**L**HEALHNHYTQKSLSLSPGK

Deimmunized D2E7 sequences with histidine mutations and traditional CH region

D2E7-SS-22 (Light Chain)

DIQMTQSPSSLSASVGDRVTITC**RASH****SIRNYL****SWY**QQKPGKAPKLLIYA**A****ASTLQ**
 GVPSRFGSGSGTDFTLTISSLQPEDVATYYC**QRYNR****APY**TFGQGTKVEIKRTVAAP
 SVFIFPPSDEQLKSGTASVCLLNNFYPRFAKVKWVDNALQSGNSQESVTEQDSKDSTY
 SLSSITLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

D2E7-SS-22 (Heavy Chain)

EVQLVESGGGLVQPGRSLRLSCAASGFTFD**HYAMH**WVRQAPGKGLEWVS**AITW**
NSGHIDYADSVEGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAK**VS****SYLSTASSL**
DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGAL
 TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
 TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
 VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
 GSFFLYSKLTVDKSRWQQGNVFSCSV**M**HEALHNHYTQKSLSLSPGK

Deimmunized D2E7 sequences with histidine mutations and mutated CH region

D2E7 SS-22 (Light Chain)

DIQMTQSPSSLSASVGDRVTITC**RASH****SIRNYL****SWY**QQKPGKAPKLLIYA**A****ASTLQ**
 GVPSRFGSGSGTDFTLTISSLQPEDVATYYC**QRYNR****APY**TFGQGTKVEIKRTVAAP
 SVFIFPPSDEQLKSGTASVCLLNNFYPRFAKVKWVDNALQSGNSQESVTEQDSKDSTY
 SLSSITLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

D2E7 SS-22 (Heavy Chain)

EVQLVESGGGLVQPGRSLRLSCAASGFTFD**HYAMH**WVRQAPGKGLEWVS**AITW**
NSGHIDYADSVEGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAK**VS****SYLSTASSL**
DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGAL
 TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
 TCPPCPAPELLGGPSVFLFPPKPKD**Q**LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
 VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
 GSFFLYSKLTVDKSRWQQGNVFSCSV**L**HEALHNHYTQKSLSLSPGK

hMAK199 (Heavy Chain)

EVQLVQSGAEVKKPGASVKVSCKAS**GYTE****FANYGII**WVRQAPGQGLEWMGWINT
YTGKPTYA**QK****FQ**GRVTMTTDTSTSTAYMELSSLRSEDTAVYYCARK**L****FTTMDV**
TDNAMDYWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK
 SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS

KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSLKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

hMAK199 (Light Chain)

*DIQMTQSPSSLSASVGDRVTITC**RASQDISOYLNWYQQKPGKAPKLLIYYTSRLQSGVPSRFSGSGSGTDFTLT**ISLQPEDFATYFC**QOQNTWPPTFGQGTKLEIKRTVAAPS**VFIFFPSDEQLKSGTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC*

hMAK199 (Heavy Chain)

*EVQLVQSGAEVKKPGASVKVSCKAS**GYTFANYGII**WVRQAPGQGLEWMGW**INTYTGKPTYAQKFQGR**VTMTTDTSTSTAYMELSSLRSEDTA VYYCARK**KLFTTMDVTDNAMDYWGQGT**TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD**QLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSLKLTVDKSRWQQGNVFCFSVL**HEALHNHYTQKSLSLSPGK*

hMAK199 (Light Chain)

*DIQMTQSPSSLSASVGDRVTITC**RASQDISOYLNWYQQKPGKAPKLLIYYTSRLQSGVPSRFSGSGSGTDFTLT**ISLQPEDFATYFC**QOQNTWPPTFGQGTKLEIKRTVAAPSVFIFFPSDEQLKSGTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC***

Key

CDR sequences: Bold underlined text

[CDR1, CDR2, and CDR3 are shown in consecutive order from N-terminus to C-terminus (e.g., RASQIRNYLS represents the VL CDR1 sequence of D2E7-SS)]

CDR mutations: Highlighted bold underlined italicized text

Variable region sequences: Regular text

Constant region sequences: Italicized text

Constant region mutations: Highlighted bold regular text

* In some embodiments, the CDR H1 is GFTFDXYAMH instead of XYAMH, where X is D or H as described above, due to an alternative and well-known antibody CDR nomenclature system. Either CDR H1 sequence annotation can be applied according to the present invention. Similarly, the hMAK199 heavy chain CDR H1 sequence shown uses the long form nomenclature system and the shorter form CDRH1 nomenclature sequence corresponding to the sequence, NYGII, is also equally applicable to the present invention.

[0043] In certain embodiments, any recombinant arabinosylated glycoprotein described herein can have the amino acid sequence of adalimumab, or an antigen-binding fragment thereof. In certain embodiments, any recombinant arabinosylated glycoprotein described herein can have an amino acid sequence of an adalimumab-like protein such as an amino acid sequence selected from the group consisting of amino acid sequences shown in Table B. In certain embodiments, any recombinant arabinosylated glycoprotein described

herein is an antibody comprising a variable light and/or variable heavy chain selected from the group consisting of variable light and/or variable heavy chains shown in Tables A and/or B. In certain embodiments, any recombinant arabinosylated glycoprotein described herein is an antibody comprising a variable light and/or heavy chain of consecutive pairs of variable light and variable heavy chains shown in Tables A and B. These embodiments are applicable to any recombinant arabinosylated glycoprotein, composition comprising a recombinant arabinosylated glycoprotein, method to produce a recombinant arabinosylated glycoprotein, or any other aspect of the present invention described herein.

[0044] The term “recombinant protein” or “recombinant polypeptide” refers to a protein molecule which is expressed from a recombinant nucleic acid molecule. The term “recombinant nucleic acid molecule” refers to a nucleic acid, such as cDNA, which is comprised of segments of DNA joined together by means of molecular biological techniques and used to engineer a cell or other protein expression system to express protein encoded therein.

[0045] The term “host cell” includes a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term “host cell” as used herein. In an embodiment, host cells include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life. In another embodiment, eukaryotic cells include protist, fungal, plant and animal cells. In another embodiment, host cells include, but are not limited to, the prokaryotic cell line *E. coli*; mammalian cell lines CHO, HEK 293, COS, NSO, SP2 and PER.C6; the insect cell line Sf9; and the fungal cell *Saccharomyces cerevisiae*.

[0046] Most peptides (or proteins, including antibodies) comprise carbohydrate or saccharide moieties attached to the peptide via specific linkages to a select number of amino acids along the length of the primary peptide chain. Such peptides or proteins can be referred to as “glycopeptides” or “glycosylated” peptides or “glycoproteins” or “glycosylated” proteins.

[0047] The oligosaccharide structure attached to the peptide chain is known as a “glycan” molecule.

[0048] The glycan structures found in glycoproteins are typically divided into two classes, “N-linked glycans” or “N-glycans” and “O-linked glycans” or “O-glycans.” Proteins and peptides expressed in eukaryotic cells typically comprise N-glycans. For example, in certain embodiments, an N-glycan is bound to an asparagine or an arginine residue in a protein via an N-acetylglucosamine residue. In certain embodiments, an N-glycan is bound to asparagine 297 (according to the number of Kabat) of an antibody, although an N-glycan can also be linked to other asparagine residues of the antibody. In certain embodiments, an N-glycan is released from a peptide or protein, such as by treatment with PNGase F. PNGase F is an enzyme that cleaves between the innermost GlcNAc and asparagine residues to liberate an N-glycan from a glycoprotein.

[0049] The predominant sugars found on glycoproteins include galactose (“Gal” or “G”), mannose (“Man”), fucose (“Fuc” or “F”), N-acetylgalactosamine (“GalNAc”), N-acetylglucosamine (“GlcNAc”) and sialic acid (e.g., N-acetylneuraminic acid (“NANA” or “NeuAc”)).

[0050] “N-linked glycosylation sites” occur in a peptide primary structure containing, for example, the amino acid sequence asparagine-X-serine/threonine, where X is any amino acid residue except proline and aspartic acid.

[0051] The term “glycoform” refers an isoform of a protein (e.g., an antibody) that differs only with respect to the number and/or type of attached glycan(s). A composition comprising a glycoprotein often includes of a number of different glycoforms.

[0052] The term “glycosylation profile” refers to the spectrum, including the amount and/or type, of N-glycans present on a protein or peptide or population of glycoforms in a glycoprotein composition. In certain embodiments, a glycosylation profile for a glycoprotein composition includes a percentage or a value for an N-glycan species relative to total N-glycans on, for example, a mol/mol basis. In certain embodiments, a percentage for an N-glycan species (e.g., HM5, G0F-GlcNAc, G0A-GlcNAc, G0, G0F, G1F, G2F, G0A, G1A, G2A) refers to the number of moles of that particular N-glycan species relative to total moles of N-glycans in a composition.

[0053] As used herein, a “modulated glycosylation profile” includes a profile of a composition comprising a protein (e.g., an antibody) which is modulated as compared to the glycosylation profile of a composition comprising that same protein produced under different conditions. In certain embodiments, a modulated glycosylation profile includes an overall increase in the level of arabinosylated N-glycans in the composition. In certain

embodiments, a modulated glycosylation profile includes an overall decrease in the level of fucosylated N-glycans in the composition. In certain embodiments, a modulated glycosylation profile includes an overall decrease in the level of mannosylation of N-glycans in the composition. In certain embodiments, a modulated glycosylation profile includes an overall decrease in the level of high mannose N-glycans in the composition. Differences in the monosaccharide composition that comprise an N-glycan are termed “microheterogeneity.” Differences in the N-glycan occupancy across the respective glycosylation sites of a protein are termed “macroheterogeneity.”

[0054] N-glycans have a common pentasaccharide core of $\text{Man}_3\text{GlcNAc}_2$. The pentasaccharide core is also referred to as a “trimannose core” or a “paucimannose core.”

[0055] N-glycans differ with respect to the presence of, and/or in the number of, branches (also called “antennae”) comprising peripheral sugars such as Gal, GalNAc, GlcNAc, NeuAc, and Fuc that are added to the $\text{Man}_3\text{GlcNAc}_2$ core structure. Optionally, this structure may also contain a core fucose molecule and/or a xylose molecule. For a review of standard glycobiology nomenclature, see Essentials of Glycobiology, Varki et al. eds., 1999, CSHL Press, the contents of which are incorporated herein by reference.

[0056] N-glycans are classified according to their branched constituents (e.g., high mannose, complex, or hybrid).

[0057] A “high mannose N-glycan” has five or more mannose residues. For example, a high mannose N-glycan having five (5) mannose residues is referred to as “HM5”; a high mannose N-glycan having six (6) mannose residues is referred to as “HM6”; a high mannose N-glycan having seven (7) mannose residues is referred to as “HM7”; a high mannose N-glycan having eight (8) mannose residues is referred to as “HM8”; and a high mannose N-glycan having nine (9) mannose residues is referred to as “HM9.”

[0058] A “complex N-glycan” typically has at least one GlcNAc residue attached to the 1,3 mannose arm and at least one GlcNAc residue attached to the 1,6 mannose arm of a pentasaccharide core. Complex-type N-glycans may also have Gal residues or GalNAc residues that are optionally modified with sialic acid or derivatives. Complex N-glycans may also have intrachain substitutions comprising “bisecting” GlcNAc residues, and a core fucose residue. Complex N-glycans may also have multiple antennae on the pentasaccharide core and are, therefore, also referred to as “multiple antennary-type glycans.”

[0059] Complex N-glycans may contain zero (G0), one (G1) or two (G2) galactose residues, as well as one fucose attached to the first GlcNAc residue on the reducing end (denoted as G0F, G1F, G2F, respectively).

[0060] The term “G0” refers the N-glycan having a $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2$ structure, wherein no terminal sialic acids or terminal Gal residues are present. See Figure 1.

[0061] The term “G0F” refers the N-glycan having a $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2$ structure plus one fucose residue attached to the first GlcNAc on the reducing end, wherein no terminal sialic acids or terminal Gal residues are present. See Figure 1.

[0062] The term “G1” refers the N-glycan having a $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2$ structure plus one terminal galactose residue. See Figure 1.

[0063] The term “G1F” refers the N-glycan having a $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2$ structure plus one terminal galactose residue and one fucose residue attached to the first GlcNAc residue on the reducing end. See Figure 1.

[0064] The term “G2” refers the N-glycan having a $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2$ structure plus two terminal galactose residues. See Figure 1.

[0065] The term “G2F” refers the N-glycan having a $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2$ structure plus two terminal galactose residues and one fucose residue attached to the first GlcNAc residue on the reducing end. See Figure 1.

[0066] As used herein, the term “G0A” refers the N-glycan having a $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2$ structure plus one arabinose residue attached to the first GlcNAc on the reducing end, wherein no terminal sialic acids or terminal Gal residues are present. See Figure 1.

[0067] As used herein, the term “G1A” refers the N-glycan having a $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2$ structure plus one terminal galactose residue and one arabinose residue attached to the first GlcNAc on the reducing end. See Figure 1.

[0068] As used herein, the term “G2A” refers the N-glycan having a $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2$ structure plus two terminal galactose residues and one arabinose residue attached to the first GlcNAc on the reducing end. See Figure 1.

[0069] A “hybrid N-glycan” comprises at least one GlcNAc on the terminal of the 1,3 mannose arm of the pentasaccharide core and zero or more mannoses on the 1,6 mannose arm of the trimannose core. N-glycans having only one terminal GlcNAc residue can be referred to as “minus GlcNAc”, which is represented by appending “-GlcNAc” to the glycan name. See Figure 1.

[0070] The term “G0F-GlcNAc” refers the N-glycan having a Man₃GlcNAc₂ core structure plus only one terminal GlcNAc residue and one fucose residue attached to the first GlcNAc on the reducing end, wherein no terminal sialic acids or terminal Gal residues are present. See Figure 1.

[0071] As used herein, the term “G0A-GlcNAc” refers the N-glycan having a Man₃GlcNAc₂ core structure plus only one terminal GlcNAc residue and one arabinose residue attached to the first GlcNAc on the reducing end, wherein no terminal sialic acids or terminal Gal residues are present. See Figure 1.

[0072] The term “G1F-GlcNAc” refers the N-glycan having a Man₃GlcNAc₂ core structure plus only one terminal GlcNAc residue and one fucose residue attached to the first GlcNAc residue on the reducing end. See Figure 1.

[0073] As used herein, the term “G1A-GlcNAc” refers the N-glycan having a Man₃GlcNAc₂ core structure plus only one terminal GlcNAc residue and one arabinose residue attached to the first GlcNAc residue on the reducing end. See Figure 1.

[0074] The term “fucosylation” or “fucosylated” refers to the presence of a fucose residue within an N-linked glycan.

[0075] The term “arabinosylation” or “arabinosylated” refers to the presence of an arabinose residue within an N-linked glycan. Arabinosylation can be achieved in numerous ways, such as through supplementation of cell culture media with arabinose (e.g., D-arabinose and/or D-altrose) or through chemical modification.

[0076] The term “core fucosylation” or “core fucosylated” refers to a fucose residue attached to the first GlcNAc on the reducing end of an N-linked glycan.

[0077] The term “core arabinosylation” or “core arabinosylated” refers to an arabinose residue attached to the first GlcNAc on the reducing end of an N-linked glycan.

[0078] Arabinose can exist in acyclic (*i.e.*, linear or open-chain) form, or in cyclic form. More than one constitutional isomeric form is contemplated for cyclic arabinose, including the 5-membered ring arabinofuranose and the 6-membered ring arabinopyranose. Furthermore, cyclic arabinose can include more than one stereochemical configuration at the anomeric carbon, the alpha or beta anomer of the aldose sugar.

[0079] Arabinose modifications or derivatives are also contemplated as arabinose residues, and are well known in the art (see, for example U.S. Pat. Nos. 8,487,110 and 6,462,191 and U.S. Pat. Publ. No. 2003/0186402). Modifications to arabinose that are contemplated for use in the invention include protection of hydroxyl (-OH) group. In

certain embodiments, the hydroxyl group is protected, thereby forming an ether (*e.g.*, alkyl ether, aralkyl ether, aryl ether, silyl ether, alkoxyalkyl ether), an ester (*e.g.*, acetate, perfluoroacetate, alkyl acetate, aralkyl acetate, aryl acetate), sulfonate, carbonate, carbamate, and the like. When valence and geometry permit, two hydroxyl groups (*e.g.*, two adjacent hydroxyl groups) can be protected together, as in a cyclic acetal or ketal. In certain embodiments, the arabinose derivative can include an aza-sugar. In example embodiments, an amino group (*e.g.*, -NH₂, -NH(alkyl), -NH(alkyl)₂) replaces a hydroxyl group of arabinose, or -NH- replaces -O-.

[0080] In certain embodiments, an arabinose residue is functionalized (*i.e.*, modified at any substitutable position) by covalent attachment of a pharmacophore, either directly, or indirectly such as via a linker. The pharmacophore can be a residue of a therapeutically active agent, a prodrug, an antibody, and the like. In embodiments in which the pharmacophore is a residue of a therapeutically active agent, the pharmacophore can be essentially the therapeutically active agent, covalently linked to the arabinose. In other embodiments, the pharmacophore relates to the parent structure of the therapeutically active agent via truncation at, for example, any one or more hydrolyzable bonds (*e.g.*, an ester bond) present in the parent structure of the therapeutically active agent. In certain embodiments, the pharmacophore can include a core structure or scaffold of a therapeutically active compound, and may include one or more derivatizations or functionalizations.

[0081] Substitutable positions of arabinose residue at which the arabinose residue can be functionalized, *e.g.*, by the pharmacophore include the oxygen of any hydroxyl group present in arabinose, any methylene carbon, or any methine carbon of arabinose. In alternative embodiments, a hydroxyl group of arabinose is replaced by a pharmacophore, optionally bound to the arabinose through a linking moiety.

[0082] The pharmacophore can be directly covalently linked (*i.e.*, directly bonded) to the arabinose residue, or alternatively can be covalently linked to the arabinose residue through a linking moiety. Exemplary linking moieties include alkylene groups, alkenylene groups, alkynylene groups, ethers, esters, amides, amines, polymeric fragments (*e.g.*, polyethyleneimine, polyethyleneoxide), heterocyclic moieties, and so forth. In certain embodiments, a pharmacophore can be linked to an arabinose residue via click chemistry (*e.g.*, alkyne-azide cycloaddition reaction), esterification, amidation, amination, and other bond-forming reactions.

[0083] In one embodiment, the pharmacophore is a residue of a therapeutically active agent that is known to increase ADCC and/or maintain (preserve) or increase complement activation. The pharmacophore can be any number of well known ADCC and/or complement activation modulatory agents, such as small molecules, proteins, nucleic acids, antibodies, and the like. For example, the pharmacophore can be a residue of taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, maytansinoids, and analogs or homologs thereof. In certain embodiments, the pharmacophore is a residue of aminoglutethimide, amsacrine, anastrozole, asparaginase, bcg, bicalutamide, bleomycin, bortezomib, buserelin, busulfan, camptothecin, capecitabine, carboplatin, carfilzomib, carmustine, chlorambucil, chloroquine, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, demethoxyviridin, dexamethasone, dichloroacetate, dienestrol, diethylstilbestrol, docetaxel, doxorubicin, epirubicin, estradiol, estramustine, etoposide, everolimus, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil and 5-fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, ironotecan, lenalidomide, letrozole, leucovorin, leuprolide, levamisole, lomustine, lonidamine, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, metformin, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, nocodazole, octreotide, oxaliplatin, paclitaxel, pamidronate, pentostatin, perfosine, plicamycin, pomalidomide, porfimer, procarbazine, raltitrexed, rituximab, romidepsin, sorafenib, streptozocin, sunitinib, suramin, tamoxifen, temozolomide, temsirolimus, teniposide, testosterone, thalidomide, thioguanine, thiotepa, titanocene dichloride, topotecan, trastuzumab, tretinoin, vinblastine, vincristine, vindesine, vinorelbine, and vorinostat (SAHA).

[0084] Arabinosylated glycopolypeptides, such as arabinosylated antibodies or antigen-binding fragments thereof, can comprise one or more than one pharmacophores of interest. For example, pharmacophore loaded arabinosylated glycopolypeptides can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more, such as 8, or any range in between, pharmacophores conjugated to the polypeptide depending upon designated linker chemistry, linker structure, linker number, functionalized positions of

arabinose, and the like. Compositions and methods for producing such pharmacophore-conjugated arabinosylated glycopolypeptides are well known in the art and include, for example, PCT Publ. No. WO 2014/152199.

[0085] The term “acyl” is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)-, preferably alkylC(O)-. “Acetate” refers to hydrocarbylC(O)O-.

[0086] The term “alkoxy” refers to an alkyl group, preferably a lower alkyl group, having an oxygen attached thereto. Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy and the like.

[0087] The term “alkoxyalkyl” refers to an alkyl group substituted with an alkoxy group and may be represented by the general formula alkyl-O-alkyl.

[0088] The term “alkenyl”, as used herein, refers to an aliphatic group containing at least one double bond and is intended to include both "unsubstituted alkenyls" and "substituted alkenyls", the latter of which refers to alkenyl moieties having substituents replacing a hydrogen on one or more carbons of the alkenyl group. Such substituents may occur on one or more carbons that are included or not included in one or more double bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed below, except where stability is prohibitive. For example, substitution of alkenyl groups by one or more alkyl, carbocyclyl, aryl, heterocyclyl, or heteroaryl groups is contemplated.

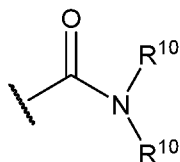
[0089] An “alkyl” group is a straight chain or branched non-aromatic hydrocarbon moiety which is completely saturated. Typically, a straight chain or branched alkyl group has from 1 to about 20 carbon atoms, preferably from 1 to about 10 unless otherwise defined. Examples of straight chained and branched alkyl groups include methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, pentyl, hexyl, pentyl and octyl. A C₁-C₆ straight chained or branched alkyl group is also referred to as a "lower alkyl" group.

[0090] The term “alkylamino”, as used herein, refers to an amino group substituted with at least one alkyl group.

[0091] The term “alkynyl”, as used herein, refers to an aliphatic group containing at least one triple bond and is intended to include both "unsubstituted alkynyls" and "substituted alkynyls", the latter of which refers to alkynyl moieties having substituents replacing a hydrogen on one or more carbons of the alkynyl group. Such substituents may occur on one or more carbons that are included or not included in one or more triple bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed

above, except where stability is prohibitive. For example, substitution of alkynyl groups by one or more alkyl, carbocyclyl, aryl, heterocyclyl, or heteroaryl groups is contemplated.

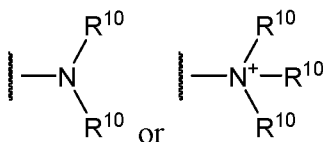
[0092] The term “amide”, as used herein, refers to a group represented by



[0093]

[0094] wherein each R^{10} independently represents a hydrogen or hydrocarbyl group, or two R^{10} are taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

[0095] The terms “amine” and “amino” are art-recognized and refer to both unsubstituted and substituted amines and salts thereof, e.g., a moiety represented by



[0096]

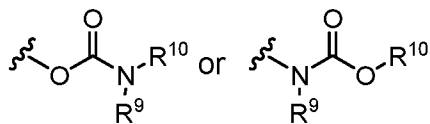
[0097] wherein each R^{10} independently represents a hydrogen or a hydrocarbyl group, or two R^{10} are taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure. In certain embodiments, amine encompasses cyclic amines, including bicyclic amines. In certain embodiments, amine includes DABCO (1,4-diazabicyclo[2.2.2]octane).

[0098] The term “aminoalkyl”, as used herein, refers to an alkyl group substituted with an amino group.

[0099] The term “aralkyl”, as used herein, refers to an alkyl group substituted with an aryl group.

[00100] The term “aryl” as used herein include substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon. Preferably the ring is a 5- to 7-membered ring, more preferably a 6-membered ring. The term “aryl” also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Aryl groups include benzene, naphthalene, phenanthrene, phenol, aniline, and the like.

[00101] The term “carbamate” is art-recognized and refers to a group represented by



[00102]

[00103] wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl group, such as an alkyl group, or R^9 and R^{10} taken together with the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

[00104] The term “carbonate” is art-recognized and refers to a group $-\text{OCO}_2\text{-}R^{10}$, wherein R^{10} represents a hydrocarbyl group.

[00105] The term “carboxy”, as used herein, refers to a group represented by the formula $-\text{CO}_2\text{H}$.

[00106] The term “ester”, as used herein, refers to a group $-\text{C}(\text{O})\text{OR}^{10}$ wherein R^{10} represents a hydrocarbyl group.

[00107] The term “ether”, as used herein, refers to a hydrocarbyl group linked through an oxygen to another hydrocarbyl group. Accordingly, an ether substituent of a hydrocarbyl group may be hydrocarbyl-O-. Ethers may be either symmetrical or unsymmetrical. Examples of ethers include, but are not limited to, heterocycle-O-heterocycle and aryl-O-heterocycle. Ethers include “alkoxyalkyl” groups, which may be represented by the general formula alkyl-O-alkyl.

[00108] The terms “heteroaryl” and “hetaryl” include substituted or unsubstituted aromatic single ring structures, preferably 5- to 7-membered rings, more preferably 5- to 6-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms “heteroaryl” and “hetaryl” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryl, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, and pyrimidine, and the like.

[00109] The term “heteroatom” as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, and sulfur.

[00110] The terms “heterocyclyl”, “heterocycle”, and “heterocyclic” refer to substituted or unsubstituted non-aromatic ring structures, preferably 3- to 10-membered rings, more preferably 3- to 7-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms.

The terms “heterocyclyl” and “heterocyclic” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heterocyclic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heterocyclyl groups include, for example, piperidine, piperazine, pyrrolidine, morpholine, lactones, lactams, and the like.

[00111] The term “heterocyclylalkyl”, as used herein, refers to an alkyl group substituted with a heterocycle group.

[00112] The term “hydrocarbyl”, as used herein, refers to a group that is bonded through a carbon atom that does not have a =O or =S substituent, and typically has at least one carbon-hydrogen bond and a primarily carbon backbone, but may optionally include heteroatoms. Thus, groups like methyl, ethoxyethyl, 2-pyridyl, and trifluoromethyl are considered to be hydrocarbyl for the purposes of this application, but substituents such as acetyl (which has a =O substituent on the linking carbon) and ethoxy (which is linked through oxygen, not carbon) are not. Hydrocarbyl groups include, but are not limited to aryl, heteroaryl, carbocycle, heterocyclyl, alkyl, alkenyl, alkynyl, and combinations thereof.

[00113] The term “hydroxyalkyl”, as used herein, refers to an alkyl group substituted with a hydroxy group.

[00114] The term “silyl” refers to a silicon moiety with three hydrocarbyl moieties attached thereto.

[00115] B. GLYCOSYLATED RECOMBINANT PROTEINS AND PROTEIN COMPOSITIONS

[00116] In one aspect, the present invention provides a recombinant glycoprotein comprising at least one N-linked glycan, wherein the N-linked glycan comprises at least one arabinose residue. The protein for which arabinosylated glycosylation is produced is not limiting and includes, for example, peptides, transmembrane proteins, antibodies, cell surface receptors, and the like, comprising an N-glycan. In certain embodiments, any recombinant arabinosylated glycoprotein described herein can have the amino acid sequence of adalimumab, or an antigen-binding fragment thereof. In certain embodiments, any recombinant arabinosylated glycoprotein described herein can have an amino acid sequence of any amino acid sequence shown in Tables A and/or B. In certain embodiments, any recombinant arabinosylated glycoprotein described herein is an antibody comprising a

variable light and/or variable heavy chain selected from the group consisting of variable light and/or variable heavy chains shown in Tables A and/or B. In certain embodiments, any recombinant arabinosylated glycoprotein described herein is an antibody comprising a variable light and/or heavy chain of consecutive pairs of variable light and variable heavy chains shown in Tables A and B. These embodiments are applicable to any recombinant arabinosylated glycoprotein, composition comprising a recombinant arabinosylated glycoprotein, method to produce a recombinant arabinosylated glycoprotein, or any other aspect of the present invention described herein.

[00117] Numerous embodiments are described herein and pair-wise, triplet, and higher order combinations thereof are further contemplated. In certain embodiments, the N-linked glycan is selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc. In certain embodiments, the at least one arabinose residue is attached to a core GlcNAc residue of the N-linked glycan. In certain embodiments, the recombinant glycoprotein does not comprise a fucose residue (e.g., the recombinant glycoprotein does not comprise a fucose residue attached to a core GlcNAc residue of the N-linked glycan).

[00118] In certain embodiments, the recombinant glycoprotein is an antibody or antigen-binding fragment thereof (e.g., a monoclonal antibody or antigen-binding fragment thereof). In certain embodiments, the glycoprotein is an anti-TNF α antibody or antigen-binding fragment thereof.

[00119] In another aspect, the present invention provides a composition comprising a population of one or more glycoforms of a recombinant glycoprotein, wherein at least one of the one or more glycoforms in the composition comprises at least one N-linked glycan, wherein the N-linked glycan comprises at least one arabinose residue. The protein for which arabinosylated glycosylation is produced is not limiting and includes, for example, peptides, transmembrane proteins, antibodies, cell surface receptors, and the like, comprising an N-glycan.

[00120] Numerous embodiments are described herein and pair-wise, triplet, and higher order combinations thereof are further contemplated. In certain embodiments, the N-linked glycan is selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc. In certain embodiments, the arabinose residue is attached to a core GlcNAc residue of the N-linked glycan. In certain embodiments, at least one of the one or more glycoforms in the composition does not comprise a fucose residue. In certain embodiments, the at least one of the one or more glycoforms in the composition does not comprise a

fucose residue attached to a core GlcNAc residue of the N-linked glycan. In certain embodiments, at least one of the one or more glycoforms in the composition comprises an arabinose residue and does not comprise a fucose residue. In certain embodiments, the at least one of the one or more glycoforms in the composition comprises at least one arabinose residue attached to a core GlcNAc residue of the N-linked glycan and does not comprise a fucose residue at the position of an arabinose attached to the core GlcNAc residue of the N-linked glycan

[00121] In certain embodiments, the glycoprotein is an antibody or antigen-binding fragment thereof (e.g., a monoclonal antibody or antigen-binding fragment thereof). In certain embodiments, the at least one glycoform is an isoform of an antibody or antigen-binding fragment thereof. In certain embodiments, the at least one glycoform is an isoform of an anti-TNF α antibody or antigen-binding fragment thereof.

[00122] In certain embodiments, the composition is a pharmaceutical composition.

[00123] In certain embodiments, at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 99%, or more, such as at least about 32%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue. In certain embodiments, at least about 32% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue.

[00124] In certain embodiments, at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 99%, or more, such as at least about 32%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue attached to an N-linked glycan selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc. In certain embodiments, at least about 32% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue attached to an N-linked glycan selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc.

[00125] In certain embodiments, at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 99%, or more, such as at least about 32%, or any range in between, inclusive, of the glycoprotein in the composition, comprises a glycoform comprising at least one arabinose residue attached to a core GlcNAc residue of the N-linked glycan and does not comprise a fucose residue at the position of an arabinose attached to the core GlcNAc residue of the N-linked glycan. In certain embodiments, at least about 32% of the glycoprotein in the composition, comprises a glycoform comprising at least one arabinose residue attached to a core GlcNAc residue of the N-linked glycan and does not comprise a fucose residue at the position of an arabinose attached to the core GlcNAc residue of the N-linked glycan.

[00126] In certain embodiments, less than about 80%, 75%, 70%, 65%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, or less, such as less than about 57%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising a fucose residue. In certain embodiments, less than about 57% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue. In certain embodiments, less than about 0.5%, or substantially 0%, of the glycoprotein in the composition comprises a glycoform comprising a fucose residue.

[00127] In certain embodiments, less than about 80%, 75%, 70%, 65%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, or less, such as less than about 57%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising a fucose residue attached to a core GlcNAc residue of the N-linked glycan. In certain embodiments, less than about 57% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue attached to a core GlcNAc residue of the N-linked glycan. In certain embodiments, less than about 0.5%, or substantially 0%, of

the glycoprotein in the composition comprises a glycoform comprising a fucose residue attached to a core GlcNAc residue of the N-linked glycan.

[00128] In certain embodiments, less than about 80%, 75%, 70%, 65%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, or less, such as less than about 7%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising five or more mannose residues. In certain embodiments, less than about 7% of the composition comprises a glycoform comprising five or more mannose residues.

[00129] In certain embodiments, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue. In certain embodiments, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, or at least about 10% of the glycoprotein in the composition comprises a glycoform comprising G0A-GlcNAc. In certain embodiments, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, or at least about 80% of the glycoprotein in the composition comprises a glycoform comprising G0A. In certain embodiments, at least about 1%, at least about 2%, at least about 3%, at least about 4%, or at least about 5% of the glycoprotein in the composition comprises a glycoform comprising G1A. In certain embodiments, at least about 0.1%, at least about 0.5%, at least about 1%, at least about 2%, or at least about 3% of the glycoprotein in the composition comprises a glycoform comprising G2A. For any of these embodiments, the glycoform can comprise at least one arabinose residue attached to a core GlcNAc residue of the N-linked glycan and does not comprise a fucose residue at the position of an arabinose attached to the core GlcNAc residue of the N-linked glycan.

[00130] In certain embodiments, less than about 60%, 50%, 40%, 30%, 20%, or 10% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue. In certain embodiments, less than about 50%, 40%, 30%, 20%, or 10% of the glycoprotein in the composition comprises a glycoform comprising G0F. In certain embodiments, substantially none of the glycoprotein in the composition comprises a glycoform comprising G0F. In certain embodiments, less than about 4%, 3%, 2%, or 1% of the glycoprotein in the composition comprises a glycoform comprising G1F. In certain

embodiments, substantially none of the glycoprotein in the composition comprises a glycoform comprising a G1F. In certain embodiments, less than about 0.5% of the glycoprotein in the composition comprises a glycoform comprising G2F. In certain embodiments, substantially none of glycoprotein in the composition comprises a glycoform comprising G2F. For any of these embodiments, the recited glycoform having less than the recited percentage in the composition comprises a fucose residue attached to a core GlcNAc residue of the N-linked glycan.

[00131] In certain embodiments, less than about 4%, 3%, 2%, or 1% of the glycoprotein in the composition comprises a glycoform comprising high mannose glycans (e.g., comprises a glycoform comprising five or more mannose residues). In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the glycoprotein in the composition comprises a glycoform comprising HM5.

[00132] In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the glycoprotein in the composition comprises a glycoform comprising high mannose glycans and less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, or less than about 10% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue.

[00133] In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the glycoprotein in the composition comprises a glycoform comprising HM5 and less than about 50%, less than about 40%, less than about 30%, less than about 20%, or less than about 10% of the glycoprotein in the composition comprises a glycoform comprising G0F.

[00134] In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the glycoprotein in the composition comprises a glycoform comprising HM5 and less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the glycoprotein in the composition comprises a glycoform comprising G1F.

[00135] In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the glycoprotein in the composition comprises a glycoform comprising HM5 and substantially none of the glycoprotein in the composition comprises a glycoform comprising G1F. In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the glycoprotein in the composition comprises a glycoform comprising HM5 and less than about 0.5% of the

glycoprotein in the composition comprises a glycoform comprising G2F. In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the glycoprotein in the composition comprises a glycoform comprising HM5 and substantially none of the glycoprotein in the composition comprises a glycoform comprising G2F.

[00136] In certain embodiments, at least about 30% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue and less than about 60% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue. In certain embodiments, at least about 40% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue and less than about 50% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue. In certain embodiments, at least about 50% of the N-glycans comprise an arabinose residue and less than about 40% of the N-glycans comprise a fucose residue. In certain embodiments, at least about 60% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue and less than about 30% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue. In certain embodiments, at least about 70% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue and less than about 20% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue. In certain embodiments, at least about 80% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue and less than about 10% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue. In certain embodiments, at least about 90% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue and less than about 1% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue.

[00137] In certain embodiments, at least about 4% of the glycoprotein in the composition comprises a glycoform comprising G0A-GlcNAc and less than about 7% of the glycoprotein in the composition comprises a glycoform comprising G0F-GlcNAc. In certain embodiments, at least about 5% of the glycoprotein in the composition comprises a glycoform comprising G0A-GlcNAc and less than about 6% of the glycoprotein in the composition comprises a glycoform comprising G0F-GlcNAc. In certain embodiments, at least about 6% of the glycoprotein in the composition comprises a glycoform comprising G0A-GlcNAc and less than about 5% of the glycoprotein in the composition comprises a

glycoform comprising G0F-GlcNAc. In certain embodiments, at least about 7% of the glycoprotein in the composition comprises a glycoform comprising G0A-GlcNAc and less than about 4% of the glycoprotein in the composition comprises a glycoform comprising G0F-GlcNAc.

[00138] In certain embodiments, at least about 40% of the glycoprotein in the composition comprises a glycoform comprising G0A and less than about 30% of the glycoprotein in the composition comprises a glycoform comprising G0F. In certain embodiments, at least about 50% of the glycoprotein in the composition comprises a glycoform comprising G0A and less than about 20% of the glycoprotein in the composition comprises a glycoform comprising G0F. In certain embodiments, at least about 60% of the glycoprotein in the composition comprises a glycoform comprising G0A and less than about 10% of the glycoprotein in the composition comprises a glycoform comprising G0F. In certain embodiments, at least about 70% of the glycoprotein in the composition comprises a glycoform comprising are G0A and less than about 1% of the glycoprotein in the composition comprises a glycoform comprising G0F.

[00139] In certain embodiments, at least about 1% of the glycoprotein in the composition comprises a glycoform comprising G1A and less than about 4% of the glycoprotein in the composition comprises a glycoform comprising G1F. In certain embodiments, at least about 2% of the glycoprotein in the composition comprises a glycoform comprising G1A and less than about 3% of the glycoprotein in the composition comprises a glycoform comprising G1F. In certain embodiments, at least about 3% of the glycoprotein in the composition comprises a glycoform comprising G1A and less than about 2% of the glycoprotein in the composition comprises a glycoform comprising G1F. In certain embodiments, at least about 4% of the glycoprotein in the composition comprises a glycoform comprising G1A and less than about 1% of the glycoprotein in the composition comprises a glycoform comprising G1F.

[00140] In certain embodiments, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% of the N-glycans comprise at least one arabinose residue. In certain embodiments, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, or at least about 10% of the N-glycans are G0A-GlcNAc. In certain embodiments, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, or at least about 80% of the N-glycans are G0A. In certain embodiments, at

least about 1%, at least about 2%, at least about 3%, at least about 4%, or at least about 5% of the N-glycans are G1A. In certain embodiments, at least about 0.1%, at least about 0.5%, at least about 1%, at least about 2%, or at least about 3% of the N-glycans are G2A.

[00141] In certain embodiments, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, or less than about 10% of the N-glycans comprise a fucose residue. In certain embodiments, less than about 50%, less than about 40%, less than about 30%, less than about 20%, or less than about 10% of the N-glycans are G0F. In certain embodiments, substantially none of the N-glycans are G0F. In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the N-glycans are G1F. In certain embodiments, substantially none of the N-glycans are G1F. In certain embodiments, less than about 0.5% of the N-glycans are G2F. In certain embodiments, substantially none of the N-glycans are G2F.

[00142] In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the N-glycans are high mannose glycans. In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the N-glycans are HM5.

[00143] In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the N-glycans are high mannose glycans and less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, or less than about 10% of the N-glycans comprise a fucose residue.

[00144] In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the N-glycans are HM5 and less than about 50%, less than about 40%, less than about 30%, less than about 20%, or less than about 10% of the N-glycans are G0F.

[00145] In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the N-glycans are HM5 and less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the N-glycans are G1F.

[00146] In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the N-glycans are HM5 and substantially none of the N-glycans are G1F. In certain embodiments, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of the N-glycans are HM5 and less than about 0.5% of the N-glycans are G2F. In certain embodiments, less than about 4%, less than about 3%, less

than about 2%, or less than about 1% of the N-glycans are HM5 and substantially none of the N-glycans are G2F.

[00147] In certain embodiments, at least about 30% of the N-glycans comprise at least one arabinose residue and less than about 60% of the N-glycans comprise a fucose residue. In certain embodiments, at least about 40% of the N-glycans comprise at least one arabinose residue and less than about 50% of the N-glycans comprise a fucose residue. In certain embodiments, at least about 50% of the N-glycans comprise at least one arabinose residue and less than about 40% of the N-glycans comprise a fucose residue. In certain embodiments, at least about 60% of the N-glycans comprise at least one arabinose residue and less than about 30% of the N-glycans comprise a fucose residue. In certain embodiments, at least about 70% of the N-glycans comprise at least one arabinose residue and less than about 20% of the N-glycans comprise a fucose residue. In certain embodiments, at least about 80% of the N-glycans comprise at least one arabinose residue and less than about 10% of the N-glycans comprise a fucose residue. In certain embodiments, at least about 90% of the N-glycans comprise at least one arabinose residue and less than about 1% of the N-glycans comprise a fucose residue.

[00148] In certain embodiments, at least about 4% of the N-glycans are G0A-GlcNAc and less than about 7% of the N-glycans are G0F-GlcNAc. In certain embodiments, at least about 5% of the N-glycans are G0A-GlcNAc and less than about 6% of the N-glycans are G0F-GlcNAc. In certain embodiments, at least about 6% of the N-glycans are G0A-GlcNAc and less than about 5% of the N-glycans are G0F-GlcNAc. In certain embodiments, at least about 7% of the N-glycans are G0A-GlcNAc and less than about 4% of the N-glycans are G0F-GlcNAc.

[00149] In certain embodiments, at least about 40% of the N-glycans are G0A and less than about 30% of the N-glycans are G0F. In certain embodiments, at least about 50% of the N-glycans are G0A and less than about 20% of the N-glycans are G0F. In certain embodiments, at least about 60% of the N-glycans are G0A and less than about 10% of the N-glycans are G0F. In certain embodiments, at least about 70% of the N-glycans are G0A and less than about 1% of the N-glycans are G0F.

[00150] In certain embodiments, at least about 1% of the N-glycans are G1A and less than about 4% of the N-glycans are G1F. In certain embodiments, at least about 2% of the N-glycans are G1A and less than about 3% of the N-glycans are G1F. In certain embodiments, at least about 3% of the N-glycans are G1A and less than about 2% of the N-

glycans are G1F. In certain embodiments, at least about 4% of the N-glycans are G1A and less than about 1% of the N-glycans are G1F.

[00151] In certain embodiments, the composition comprising recombinant glycoprotein glycoforms exhibits a modulated glycosylation profile compared to a control composition.

[00152] In certain embodiments, the modulated glycosylation profile includes a differing type of glycan species. For example, in certain embodiments, the recombinant glycoprotein composition comprises N-glycans having at least one arabinose residue whereas the control composition lacks N-glycans having at least one arabinose residue. As another example, in certain embodiments, the glycoprotein composition lacks N-glycans having a core fucose residue, whereas the control composition includes N-glycans having a core fucose residue.

[00153] In certain embodiments, the modulated glycosylation profile includes a differing amount or level of glycan species. For example, in certain embodiments, the amount or level of fucosylation is decreased. In certain embodiments, the level of G0F-GlcNAc species is decreased. In certain embodiments, the level of G0F-GlcNAc species is decreased by at least about 1%; by at least about 5%; by at least about 10%; or by about 10%. In certain embodiments, the level of G0F species is decreased. In certain embodiments, the level of G0F species is decreased by at least about 1%; by at least about 10%; by at least about 25%; by at least about 50%; by at least about 75%; by about 25%; or by about 75%. In certain embodiments, the level of G1F species is decreased. In certain embodiments, the level of G1F species is decreased by at least about 1%; by at least about 5%; by at least about 10%; or by about 5%. In certain embodiments, the level of G2F species is decreased. In certain embodiments, the level of G2F species is decreased by at least about 0.1%; by at least about 1%; by about 0.5%. All decreases are relative to the control composition.

[00154] As another example, in certain embodiments, the amount or level of mannosylation is decreased. In certain embodiments, the level of Man5 species is decreased. In certain embodiments, the level of Man5 species is decreased by at least about 0.1%; by at least about 1%; by at least about 2%; by at least about 3%; by at least about 4%; or by about 1%. In certain embodiments, the level of Man6 species is decreased. In certain embodiments, the level of Man6 species is decreased by at least about 0.1%; or by about 0.5%. In certain embodiments, the level of Man7 species is decreased. In certain

embodiments, the level of Man8 species is decreased. All decreases are relative to the control composition.

[00155] As yet another example, in certain embodiments, the amount or level of arabinosylation is increased. In certain embodiments, the level of G0A-GlcNAc species is increased. In certain embodiments, the level of G0A species is increased. In certain embodiments, the level of G1A species is increased. In certain embodiments, the level of G2A species is increased. All increases are relative to the control composition.

[00156] In certain embodiments, the recombinant glycoprotein composition is prepared by (a) providing a mammalian host cell that expresses a protein containing N-linked glycans; and (b) culturing the host cell in a cell culture media containing D-arabinose and/or D-altrose. In certain embodiments, the cell culture media is supplemented with D-arabinose and/or D-altrose.

[00157] In certain embodiments, the control composition is prepared by (a) providing a mammalian host cell that expresses a protein containing N-linked glycans; and (b) culturing the host cell in the absence of D-arabinose and/or D-altrose. In certain embodiments, the control composition is prepared by (a) providing a mammalian host cell that expresses a protein containing N-linked glycans; and (b) culturing the host cell without D-arabinose and/or D-altrose supplementation. In any process described herein, the method can use D-arabinose alone, D-altrose alone, or the combination of D-arabinose and D-altrose.

[00158] In certain embodiments, the same type of host cell is used to prepare the glycoprotein composition and the control composition. In certain embodiments, the host cell is a rodent cell. In certain embodiments, the host cell is a CHO cell. In certain embodiments, the same cell culture media is used to prepare the glycoprotein composition and the control composition.

[00159] In certain embodiments, the at least one glycoform is an isoform of an antibody or antigen-binding fragment thereof. In certain embodiments, the at least one glycoform is an isoform of an anti-TNF α antibody or antigen-binding fragment thereof.

[00160] In certain embodiments, the modulated glycosylation profile includes a differing type of glycan species. For example, in certain embodiments, the glycoprotein composition comprises N-glycans having an arabinose residue whereas the control composition lacks N-glycans having an arabinose residue. As another example, in certain

embodiments, the glycoprotein composition lacks N-glycans having a core fucose residue whereas the control composition includes N-glycans having a core fucose residue.

[00161] As can be appreciated from the present disclosure, compositions comprising varying levels of glycoforms of a protein such as a human antibody, or antigen-binding fragment thereof, are useful in that one or more desired characteristics, e.g., rate of serum clearance or ADCC activity, may be achieved by varying the glycoform compositions.

[00162] In one aspect, the present invention provides methods for producing a recombinant glycoprotein or a glycoprotein composition, such as a recombinant glycoprotein or glycoprotein composition described herein. In certain embodiments, the methods comprise supplementing a cell culture media with arabinose, such as D-arabinose and/or D-altrose. In certain embodiments, the methods comprise culturing a host cell expressing the glycoprotein in a cell culture media supplemented with D-arabinose and/or D-altrose. In certain embodiments, the host cell is a mammalian cell. In certain embodiments, the mammalian cell is a rodent cell. In certain embodiments, the host cell is a CHO cell. In certain embodiments, the cell culture media is supplemented with about 1 mM, about 5 mM, about 10 mM, about 15 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, or about 100 mM D-arabinose and/or D-altrose. In certain embodiments, the cell culture media is supplemented with about 10 mM D-arabinose and/or D-altrose. In certain embodiments, the cell culture media is supplemented with about 15 mM D-arabinose and/or D-altrose.

[00163] In certain embodiments, the glycoprotein produced by the methods described herein is an arabinosylated glycoprotein as described herein. For example, in certain embodiments, the arabinosylated glycoprotein is an arabinosylated antibody or antigen-binding fragment thereof. In certain embodiments, the arabinosylated glycoprotein is an anti-TNF α antibody or antigen-binding fragment thereof.

[00164] In certain embodiments, the arabinosylated glycoprotein comprises an arabinose residue attached to a core GlcNAc residue. In certain embodiments, the arabinosylated glycoprotein comprises an N-glycan selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc. In certain embodiments, the arabinosylated glycoprotein lacks a core fucose residue.

[00165] In certain embodiments, the glycoprotein composition produced by the methods described herein comprises at least one glycoform having an N-linked glycan comprising arabinose. In certain embodiments, the N-linked glycan comprises an arabinose

residue attached to a core GlcNAc residue. In certain embodiments, the N-linked glycan is G0A, G1A, G2A, G0A-GlcNAc, or G1A-GlcNAc. In certain embodiments, the N-linked glycan lacks a core fucose residue.

[00166] In certain embodiments, the at least one glycoform is an isoform of an antibody or antigen-binding fragment thereof. In certain embodiments, the at least one glycoform is an isoform of an anti-TNF α antibody or antigen-binding fragment thereof.

[00167] In certain embodiments, the glycoprotein composition produced by the methods described herein exhibits a modulated glycosylation profile compared to a control composition. For example, in certain embodiments, the methods described herein can be used to produce a hypofucosylated composition having decreased amounts or levels of fucose residues relative to a control composition. Such altered glycosylation patterns have previously been demonstrated to increase the ADCC ability of antibodies. As another example, in certain embodiments, the methods described herein can be used to produce a hypomannosylated composition having decreased amounts or levels of mannose residues relative to a control composition. Such altered glycosylation patterns have previously been demonstrated to reduce immunogenicity and/or improve pharmacokinetic properties of antibodies.

[00168] In certain embodiments, the modulated glycosylation profile includes a differing type of glycan species. For example, in certain embodiments, the glycoprotein composition comprises N-glycans having an arabinose residue whereas the control composition lacks N-glycans having an arabinose residue. As another example, in certain embodiments, the glycoprotein composition lacks N-glycans having a core fucose residue whereas the control composition includes N-glycans having a core fucose residue.

[00169] In certain embodiments, the modulated glycosylation profile includes a differing amount or level of glycan species. For example, in certain embodiments, the amount or level of fucosylation is decreased. In certain embodiments, the level of G0F-GlcNAc species is decreased. In certain embodiments, the level of G0F-GlcNAc species is decreased by at least about 1%; by at least about 5%; by at least about 10%; or by about 10%. In certain embodiments, the level of G0F species is decreased. In certain embodiments, the level of G0F species is decreased by at least about 1%; by at least about 10%; by at least about 25%; by at least about 50%; by at least about 75%; by about 25%; or by about 75%. In certain embodiments, the level of G1F species is decreased. In certain embodiments, the level of G1F species is decreased by at least about 1%; by at least about

5%; by at least about 10%; or by about 5%. In certain embodiments, the level of G2F species is decreased. In certain embodiments, the level of G2F species is decreased by at least about 0.1%; by at least about 1%; or by about 0.5%. All decreases are relative to a control composition.

[00170] As another example, in certain embodiments, the amount or level of mannosylation is decreased. In certain embodiments, the level of Man5 species is decreased. In certain embodiments, the level of Man5 species is decreased by at least about 0.1%; by at least about 1%; by at least about 2%; by at least about 3%; by at least about 4%; or by about 1%. In certain embodiments, the level of Man6 species is decreased. In certain embodiments, the level of Man6 species is decreased by at least about 0.1%; or by about 0.5%. In certain embodiments, the level of Man7 species is decreased. In certain embodiments, the level of Man8 species is decreased. All decreases are relative to a control composition.

[00171] As yet another example, in certain embodiments, the amount or level of arabinosylation is increased. In certain embodiments, the level of G0A-GlcNAc species is increased. In certain embodiments, the level of G0A species is increased. In certain embodiments, the level of G1A species is increased. In certain embodiments, the level of G2A species is increased. All increases are relative to a control composition.

[00172] In certain embodiments, the control composition is prepared by (a) providing a mammalian host cell that expresses a protein containing N-linked glycans; and (b) culturing the host cell in the absence of D-arabinose and/or D-altrose. In certain embodiments, the control composition is prepared by (a) providing a mammalian host cell that expresses a protein containing N-linked glycans; and (b) culturing the host cell without D-arabinose and/or without D-altrose supplementation.

[00173] In certain embodiments, the same type of host cell is used to prepare the glycoprotein composition and the control composition. In certain embodiments, the host cell is a rodent cell. In certain embodiments, the host cell is a CHO cell. In certain embodiments, the same cell culture media is used to prepare the glycoprotein composition and the control composition.

[00174] In certain embodiments, the at least one glycoform is an isoform of an antibody or antigen-binding fragment thereof. In certain embodiments, the at least one glycoform is an isoform of an anti-TNF α antibody or antigen-binding fragment thereof.

[00175] In another aspect, the present invention provides methods for producing a glycoprotein composition having a low amount of fucosylated N-glycans and/or high mannose N-glycans.

[00176] In certain embodiments, the glycoprotein composition has a low amount of core fucosylated N-glycans and/or high mannose N-glycans. In certain embodiments, the glycoprotein composition has a low amount of G1F-GlcNAc, G0F, G1F, G2F, HM5, and/or HM6. In certain embodiments, the glycoprotein composition has a low amount of G1F-GlcNAc. In certain embodiments, the glycoprotein composition has a low amount of G0F. In certain embodiments, the glycoprotein composition has a low amount of G1F. In certain embodiments, the glycoprotein composition has a low amount of G2F. In certain embodiments, the glycoprotein composition has a low amount of high mannose. In certain embodiments, the glycoprotein composition has a low amount of HM5. In certain embodiments, the glycoprotein composition has a low amount of HM6.

[00177] In certain embodiments, at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 99%, or more, such as at least about 32%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue. In certain embodiments, at least about 32% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue.

[00178] In certain embodiments, at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 99%, or more, such as at least about 32%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue attached to an N-linked glycan selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc. In certain embodiments, at least about 32% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue attached to an N-linked glycan selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc.

[00179] In certain embodiments, at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 99%, or more, such as at least about 32%, or any range in between, inclusive, of the glycoprotein in the composition, comprises a glycoform comprising at least one arabinose residue attached to a core GlcNAc residue of the N-linked glycan and does not comprise a fucose residue at the position of an arabinose attached to the core GlcNAc residue of the N-linked glycan. In certain embodiments, at least about 32% of the glycoprotein in the composition, comprises a glycoform comprising at least one arabinose residue attached to a core GlcNAc residue of the N-linked glycan and does not comprise a fucose residue at the position of an arabinose attached to the core GlcNAc residue of the N-linked glycan.

[00180] In certain embodiments, less than about 80%, 75%, 70%, 65%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, or less, such as less than about 57%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising a fucose residue. In certain embodiments, less than about 57% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue. In certain embodiments, less than about 0.5%, or substantially 0%, of the glycoprotein in the composition comprises a glycoform comprising a fucose residue.

[00181] In certain embodiments, less than about 80%, 75%, 70%, 65%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, or less, such as less than about 57%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising a fucose residue attached to a core GlcNAc residue of the N-linked glycan. In certain embodiments, less than about 57% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue attached to a core GlcNAc residue of the N-linked glycan. In certain embodiments, less than about 0.5%, or substantially 0%, of

the glycoprotein in the composition comprises a glycoform comprising a fucose residue attached to a core GlcNAc residue of the N-linked glycan.

[00182] In certain embodiments, less than about 80%, 75%, 70%, 65%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, or less, such as less than about 7%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising five or more mannose residues. In certain embodiments, less than about 7% of the composition comprises a glycoform comprising five or more mannose residues.

[00183] In certain embodiments, the glycoprotein composition includes no more than about 60%, about 50%, about 40%, about 30%, about 20%, or about 10% fucosylated N-glycans, e.g., no more than about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, about 0.5%, or about 0.1%. In certain embodiments, the glycoprotein composition includes no more than about 50%, about 40%, about 30%, about 20%, or about 10% G0F glycans. In certain embodiments, the glycoprotein composition includes no detectable G0F glycans. In certain embodiments, the glycoprotein composition includes no more than about 4%, about 3%, about 2%, or about 1% G1F glycans. In certain embodiments, the glycoprotein composition includes no G1F glycans. In certain embodiments, the glycoprotein composition includes no more than about 0.5% of the N-glycans are G2F. In certain embodiments, the glycoprotein composition includes no detectable G2F glycans. The level of fucose structures present in a glycoprotein composition can be generally measured as the level of glycans containing fucose structures relative to total amount of glycans in a sample, such as a glycoprotein preparation. In some embodiments, the level of fucose present in a glycoprotein composition can be generally reported as % abundance relative to total glycan mass.

[00184] In some embodiments, the glycoprotein composition includes no more than about 20%, about 15%, about 12%, or about 10% high mannose glycans, e.g., no more than about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, about 0.5%, or about 0.1%. In one embodiment, the glycoprotein composition has high mannose structures (e.g., has at least 0.01%, 0.05% or 0.1% high mannose) but has no more than about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, about 0.5%, or about 0.1%, e.g., has between 0.05-20% high mannose,

0.1-15% high mannose 0.1-10% high mannose, between 1-10% high mannose, between 1-5% high mannose. The level of high mannose structures present in a glycoprotein composition can be generally measured as the level of glycans containing high mannose structures relative to total amount of glycans in a sample, such as a glycoprotein preparation. In some embodiments, the level of high mannose structures present in a glycoprotein composition can be generally reported as % abundance relative to total glycan mass.

[00185] While the above described glycoprotein composition characteristics have been described according to glycan percentages, such percentages also apply to the percentage of glycoprotein represented by glycoforms comprising the recited glycan structure.

[00186] In one aspect, the present invention provides methods for producing a glycoprotein composition having a reduced fucose and/or mannose content.

[00187] In certain embodiments, the glycoprotein composition has a reduced core fucose and/or high mannose content. In certain embodiments, the glycoprotein composition has a reduced fucose and/or mannose content relative to a control composition. In certain embodiments, the glycoprotein composition has a reduced G1F-GlcNAc, G0F, G1F, G2F, HM5, and/or HM6 content. In certain embodiments, the glycoprotein composition has a reduced G1F-GlcNAc content. In certain embodiments, the glycoprotein composition has a reduced G0F content. In certain embodiments, the glycoprotein composition has a reduced G1F content. In certain embodiments, the glycoprotein composition has a reduced G2F content. In certain embodiments, the glycoprotein composition has a reduced high mannose content. In certain embodiments, the glycoprotein composition has a reduced HM5 content. In certain embodiments, the glycoprotein composition has a reduced HM6 content.

[00188] In one aspect, the present invention provides cell culture methods for obtaining a desired glycosylation profile for a glycoprotein composition.

[00189] In certain embodiments, the methods comprise supplementing a cell culture media with D-arabinose and/or D-altrose. In certain embodiments, the methods comprise culturing a host cell expressing the glycoprotein in a cell culture media supplemented with arabinose, such as D-arabinose and/or D-altrose. In certain embodiments, the host cell is a mammalian cell. In certain embodiments, the mammalian cell is a rodent cell. In certain embodiments, the host cell is a CHO cell. In certain embodiments, the cell culture media is supplemented with about 1 mM, about 5 mM, about 10 mM, about 15 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM,

about 90 mM, or about 100 mM D-arabinose and/or D-altrose. In certain embodiments, the cell culture media is supplemented with about 10 mM D-arabinose and/or D-altrose. In certain embodiments, the cell culture media is supplemented with about 15 mM D-arabinose and/or D-altrose.

[00190] In certain embodiments, at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 99%, or more, such as at least about 32%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue. In certain embodiments, at least about 32% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue.

[00191] In certain embodiments, at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 99%, or more, such as at least about 32%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue attached to an N-linked glycan selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc. In certain embodiments, at least about 32% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue attached to an N-linked glycan selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc.

[00192] In certain embodiments, at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 99%, or more, such as at least about 32%, or any range in between, inclusive, of the glycoprotein in the composition, comprises a glycoform comprising at least one arabinose residue attached to a core GlcNAc residue of the N-linked glycan and does not comprise a fucose residue at the position of an arabinose attached to the core GlcNAc residue of the N-linked glycan. In certain embodiments, at least about 32% of the glycoprotein in the composition, comprises a glycoform comprising at least one arabinose residue attached to a core GlcNAc residue of

the N-linked glycan and does not comprise a fucose residue at the position of an arabinose attached to the core GlcNAc residue of the N-linked glycan.

[00193] In certain embodiments, less than about 80%, 75%, 70%, 65%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, or less, such as less than about 57%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising a fucose residue. In certain embodiments, less than about 57% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue. In certain embodiments, less than about 0.5%, or substantially 0%, of the glycoprotein in the composition comprises a glycoform comprising a fucose residue.

[00194] In certain embodiments, less than about 80%, 75%, 70%, 65%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, or less, such as less than about 57%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising a fucose residue attached to a core GlcNAc residue of the N-linked glycan. In certain embodiments, less than about 57% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue attached to a core GlcNAc residue of the N-linked glycan. In certain embodiments, less than about 0.5%, or substantially 0%, of the glycoprotein in the composition comprises a glycoform comprising a fucose residue attached to a core GlcNAc residue of the N-linked glycan.

[00195] In certain embodiments, less than about 80%, 75%, 70%, 65%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, or less, such as less than about 7%, or any range in between, inclusive, of the glycoprotein in the composition comprises a glycoform comprising five or more mannose residues. In certain embodiments, less than about 7% of the composition comprises a glycoform comprising five or more mannose residues.

[00196] In certain embodiments, a desired glycosylation profile includes at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% arabinosylated N-glycans in a glycoprotein composition. In certain embodiments, a desired glycosylation profile includes at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, or at least about 10% G0A-GlcNAc glycans. In certain embodiments, a desired glycosylation profile includes at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, or at least about 80% G0A glycans. In certain embodiments, a desired glycosylation profile includes at least about 1%, at least about 2%, at least about 3%, at least about 4%, or at least about 5% G1A glycans. In certain embodiments, a desired glycosylation profile includes at least about 0.1%, at least about 0.5%, at least about 1%, at least about 2%, or at least about 3% G2A glycans. The level of arabinose structures present in a glycoprotein composition can be generally measured as the level of glycans containing arabinose structures relative to total amount of glycans in a sample, such as a glycoprotein preparation. In some embodiments, the level of arabinose present in a glycoprotein composition can be generally reported as % abundance relative to total glycan mass.

[00197] In certain embodiments, a desired glycosylation profile includes no more than about 60%, about 50%, about 40%, about 30%, about 20%, or about 10% fucosylated N-glycans in a glycoprotein composition, e.g., no more than about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, about 0.5%, or about 0.1%. In certain embodiments, a desired glycosylation profile includes no more than about 50%, about 40%, about 30%, about 20%, or about 10% G0F glycans. In certain embodiments, a desired glycosylation profile includes no detectable G0F glycans. In certain embodiments, a desired glycosylation profile includes no more than about 4%, about 3%, about 2%, or about 1% G1F glycans. In certain embodiments, a desired glycosylation profile includes no G1F glycans. In certain embodiments, a desired glycosylation profile includes no more than about 0.5% of the N-glycans are G2F. In certain embodiments, a desired glycosylation profile includes no detectable G2F glycans. The level of fucose structures present in a glycoprotein composition can be generally measured as the level of glycans containing fucose structures relative to total amount of glycans in a sample, such as a glycoprotein preparation. In some embodiments, the level of fucose present in a glycoprotein composition can be generally reported as % abundance relative to total glycan mass.

[00198] In some embodiments, a desired glycosylation profile includes no more than about 20%, about 15%, about 12%, or about 10% high mannose glycans in a glycoprotein composition, e.g., no more than about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, about 0.5%, or about 0.1%. In one embodiment, the glycoprotein composition has high mannose structures (e.g., has at least 0.01%, 0.05% or 0.1% high mannose) but has no more than about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, about 0.5%, or about 0.1%, e.g., has between 0.05-20% high mannose, 0.1-15% high mannose 0.1-10% high mannose, between 1-10% high mannose, between 1-5% high mannose. The level of high mannose structures present in a glycoprotein composition can be generally measured as the level of glycans containing high mannose structures relative to total amount of glycans in a sample, such as a glycoprotein preparation. In some embodiments, the level of high mannose structures present in a glycoprotein composition can be generally reported as % abundance relative to total glycan mass.

[00199] In certain embodiments, the methods allow for a glycosylation profile of a glycoprotein composition to be controlled. In certain embodiments, the methods are for controlling levels of fucosylated glycans in the glycoprotein composition. In certain embodiments, core fucosylated N-glycan levels are at or below the low limit of detection. In certain embodiments, D-arabinose and/or D-altrose completely replaces fucose within the N-glycans. In certain embodiments, the methods are for controlling levels of high mannose glycans in the glycoprotein composition. In certain embodiments, the methods are for simultaneously controlling levels of fucosylated glycans and levels high mannose glycans in a glycoprotein composition.

[00200] In certain embodiments, the methods allow for a glycosylation profile of a glycoprotein composition to be modulated relative to a control composition.

[00201] In certain embodiments, the glycosylation profile of the glycoprotein composition includes one or more different types of glycan species that is absent in the control composition. For example, in certain embodiments, the glycoprotein composition comprises N-glycans having an arabinose residue whereas the control composition lacks N-glycans having an arabinose residue. In certain embodiments, the glycosylation profile of the glycoprotein composition lacks one or more types of glycan species that is present in the control composition. For example, in certain embodiments, the glycoprotein

composition lacks N-glycans having a core fucose residue whereas the control composition includes N-glycans having a core fucose residue.

[00202] In certain embodiments, the glycosylation profile of the glycoprotein composition includes a differing amount or level of glycan species relative to the control composition. For example, in certain embodiments, the amount or level of fucosylation is decreased relative to the control composition. In certain embodiments, the level of G0F-GlcNAc species is decreased relative to the control composition. In certain embodiments, the level of G0F-GlcNAc species is decreased by at least about 1%; by at least about 5%; by at least about 10%; or by about 10% relative to the control composition. In certain embodiments, the level of G0F species is decreased relative to the control composition. In certain embodiments, the level of G0F species is decreased by at least about 1%; by at least about 10%; by at least about 25%; by at least about 50%; by at least about 75%; by about 25%; or by about 75% relative to the control composition. In certain embodiments, the level of G1F species is decreased relative to the control composition. In certain embodiments, the level of G1F species is decreased by at least about 1%; by at least about 5%; by at least about 10%; or by about 5% relative to the control composition. In certain embodiments, the level of G2F species is decreased relative to the control composition. In certain embodiments, the level of G2F species is decreased by at least about 0.1%; by at least about 1%; by about 0.5% relative to the control composition.

[00203] As another example, in certain embodiments, the amount or level of mannosylation is decreased relative to the control composition. In certain embodiments, the level of Man5 species is decreased. In certain embodiments, the level of Man5 species is decreased by at least about 0.1%; by at least about 1%; by at least about 2%; by at least about 3%; by at least about 4%; or by about 1% relative to the control composition. In certain embodiments, the level of Man6 species is decreased relative to the control composition. In certain embodiments, the level of Man6 species is decreased by at least about 0.1%; or by about 0.5% relative to the control composition. In certain embodiments, the level of Man7 species is decreased relative to the control composition. In certain embodiments, the level of Man8 species is decreased relative to the control composition.

[00204] While the above described glycoprotein composition characteristics for the aspect of the present invention have been described according to glycan percentages, such percentages also apply to the percentage of glycoprotein represented by glycoforms comprising the recited glycan structure.

[00205] As yet another example, in certain embodiments, the amount or level of arabinosylation is increased relative to the control composition. In certain embodiments, the level of G0A-GlcNAc species is increased relative to the control composition. In certain embodiments, the level of G0A species is increased relative to the control composition. In certain embodiments, the level of G1A species is increased relative to the control composition. In certain embodiments, the level of G2A species is increased relative to the control composition.

[00206] In certain embodiments, the control composition is prepared by (a) providing a mammalian host cell that expresses a protein containing N-linked glycans; and (b) culturing the host cell in the absence of D-arabinose and/or in the absence of D-altrose. In certain embodiments, the control composition is prepared by (a) providing a mammalian host cell that expresses a protein containing N-linked glycans; and (b) culturing the host cell without D-arabinose supplementation and/or without D-altrose supplementation.

[00207] In certain embodiments, the same type of host cell is used to prepare the glycoprotein composition and the control composition. In certain embodiments, the host cell is a rodent cell. In certain embodiments, the host cell is a CHO cell. In certain embodiments, the same cell culture media is used to prepare the glycoprotein composition and the control composition.

[00208] It is known to those skilled in the art that differing protein glycosylation profiles may result in differing protein characteristics. For instance, the efficacy of a therapeutic protein produced in a microorganism host, such as yeast, and glycosylated utilizing the yeast endogenous pathway may be reduced compared to that of the same protein expressed in a mammalian cell, such as a CHO cell line. Such glycoproteins may also be immunogenic in humans and show reduced half-life *in vivo* after administration. Specific receptors in humans and other animals may recognize specific glycosyl residues and promote the rapid clearance of the protein from the bloodstream. Other adverse effects may include changes in protein folding, solubility, susceptibility to proteases, trafficking, transport, compartmentalization, secretion, recognition by other proteins or factors, antigenicity, or allergenicity. Accordingly, using the methods of the invention, one of skill in the art may modulate the glycosylation profile of a protein (e.g., an antibody) to achieve a desired activity such as increased or decreased rate of clearance, maintained (preserved) or increased complement activity, and/or increased ADCC activity.

[00209] Upstream Process Technologies

[00210] In one aspect, the present invention provides methods for producing a recombinant glycoprotein (e.g., an antibody, or antigen binding fragment thereof) with a modulated glycosylation profile. In certain embodiments, the methods involve modification of the conditions used during upstream protein production, such as recombinant cell culture conditions. In certain embodiments, the methods comprise supplementing the recombinant cell culture media with D-arabinose and/or D-altrose to modulate the glycosylation profile of the protein.

[00211] As described herein, the host cell culture conditions can be modified as compared to conditions during production of the same protein without modulation of the glycosylation profile. In certain embodiments, a protein with a modulated glycosylation profile is produced by culturing cells expressing the protein in a cell culture media supplemented with D-arabinose and/or supplemented with D-altrose.

[00212] To produce a protein or protein composition with a modulated glycosylation profile, one or more DNAs encoding the protein, such as DNAs encoding partial or full-length light and heavy chains in the case of antibodies, are inserted into one or more expression vector such that the genes are operatively linked to transcriptional and translational control sequences. (See, e.g., U.S. Pat. No. 6,090,382, the entire contents of which are incorporated herein by reference.) In this context, the term “operatively linked” is intended to mean that a gene encoding the protein is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. In certain embodiments, the protein or protein composition with a modulated glycosylation profile will comprise multiple polypeptides, such as the heavy and light chains of an antibody. Thus, in certain embodiments, genes encoding multiple polypeptides, such as antibody light chain genes and antibody heavy chain genes, can be inserted into a separate vector or, more typically, the genes are inserted into the same expression vector. Genes are inserted into expression vectors by standard methods (e.g., ligation of complementary restriction sites on the gene fragment and vector, or blunt end ligation if no restriction sites are present). Prior to insertion of the gene or genes, the expression vector may already carry additional polypeptide sequences, such as, but not limited to, antibody constant region sequences. For example, one approach to converting the anti-TNF α antibody or anti-TNF α antibody-related VH and VL sequences to full-length antibody

genes is to insert them into expression vectors already encoding heavy chain constant and light chain constant regions, respectively, such that the VH segment is operatively linked to the CH segment(s) within the vector and the VL segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the protein from a host cell. The gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

[00213] In addition to protein coding genes, a recombinant expression vector can carry one or more regulatory sequence that controls the expression of the protein coding genes in a host cell. The term “regulatory sequence” is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the protein coding genes. Such regulatory sequences are described, e.g., in Goeddel; *Gene Expression Technology Methods in Enzymology* 185, Academic Press, San Diego, Calif. (1990), the entire teaching of which is incorporated herein by reference. It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Suitable regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (e.g., the adenovirus major late promoter (AdMLP)) and polyoma. For further description of viral regulatory elements, and sequences thereof, see, e.g., U.S. Pat. No. 5,168,062 by Stinski, U.S. Pat. No. 4,510,245 by Bell et al. and U.S. Pat. No. 4,968,615 by Schaffner et al., the entire teachings of all of which are incorporated herein by reference.

[00214] A recombinant expression vector may also carry one or more additional sequences, such as a sequence that regulates replication of the vector in host cells (e.g., origins of replication) and/or a selectable marker gene. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel et al., the entire teachings of both of which are incorporated herein by reference). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell

into which the vector has been introduced. Suitable selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr-host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

[00215] An antibody, or antigen binding fragment thereof, to be used in the method of preparing a protein or protein composition can be prepared by recombinant expression of immunoglobulin light and heavy chain genes in a host cell. To express an antibody recombinantly, a host cell is transfected with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and heavy chains of the antibody such that the light and heavy chains are expressed in the host cell and secreted into the medium in which the host cells are cultured, from which medium the antibodies can be recovered. Standard recombinant DNA methodologies are used to obtain antibody heavy and light chain genes, incorporate these genes into recombinant expression vectors and introduce the vectors into host cells, such as those described in Sambrook, Fritsch and Maniatis (eds), *Molecular Cloning; A Laboratory Manual*, Second Edition, Cold Spring Harbor, N.Y., (1989), Ausubel et al. (eds.) *Current Protocols in Molecular Biology*, Greene Publishing Associates, (1989) and in U.S. Pat. Nos. 4,816,397 & 6,914,128, the entire teachings of both of which are incorporated herein.

[00216] For expression of a protein, for example, the light and heavy chains of an antibody, the expression vector(s) encoding the protein is (are) transfected into a host cell by standard techniques. The various forms of the term “transfection” are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the proteins of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, such as mammalian host cells, is suitable because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active protein. Prokaryotic expression of protein genes has been reported to be ineffective for production of high yields of active protein (Boss and Wood (1985) *Immunology Today* 6:12-13, the entire teaching of which is incorporated herein by reference).

[00217] Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms,

e.g., Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescens*, and *Shigella*, as well as Bacilli such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published Apr. 12, 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One suitable *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting.

[00218] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for polypeptide encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; *Kluyveromyces* hosts such as, e.g., *K. lactis*, *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilum* (ATCC 36,906), *K. thermotolerans*, and *K. marxianus*; *Yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger*.

[00219] Suitable host cells for the expression of a protein or protein composition are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can also be utilized as hosts.

[00220] Mammalian cells can be used for expression and production of the protein compositions of the invention, however other eukaryotic cell types can also be employed in the context of the instant invention. See, e.g., Winnacker, *From Genes to Clones*, VCH Publishers, N.Y., N.Y. (1987). Suitable mammalian host cells for expressing recombinant

proteins according to the invention include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasin, (1980) PNAS USA 77:4216-4220, used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp (1982) Mol. Biol. 159:601-621, the entire teachings of which are incorporated herein by reference), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding protein genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or secretion of the antibody into the culture medium in which the host cells are grown. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TR1 cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2), the entire teachings of which are incorporated herein by reference.

[00221] Host cells are transformed with the above-described expression or cloning vectors for protein production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

[00222] The host cells used to produce a protein or a protein composition may be cultured in a variety of media which are supplemented or modified in accordance with the present invention. Commercially available media such as Ham's F10™ (Sigma), Minimal Essential Medium™ (MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium™ (DMEM), (Sigma), Iscove's Modified Dulbecco's Medium, Minimal Essential Medium-alpha. (MEM-alpha), DME/F12, alpha MEM, Basal Medium Eagle with Earle's BSS, DMEM high Glucose, with L-Glutamine, DMEM high glucose, without L-Glutamine, DMEM low Glucose, without L-Glutamine, DMEM:F12 1:1, with L-Glutamine,

GMEM (Glasgow's MEM), GMEM with L-glutamine, Grace's Complete Insect Medium, Grace's Insect Medium, without FBS, Ham's F-10, with L-Glutamine, Ham's F-12, with L-Glutamine, IMDM with HEPES and L-Glutamine, IMDM with HEPES and without L-Glutamine, IPL-41 Insect Medium, L-15 (Leibovitz)(2.times.), without L-Glutamine or Phenol Red, L-15 (Leibovitz), without L-Glutamine, McCoy's 5A Modified Medium, Medium 199, MEM Eagle, without L-Glutamine or Phenol Red (2.times.), MEM Eagle-Earle's BSS, with L-glutamine, MEM Eagle-Earle's BSS, without L-Glutamine, MEM Eagle-Hanks BSS, without L-Glutamine, NCTC-109, with L-Glutamine, Richter's CM Medium, with L-Glutamine, RPMI 1640 with HEPES, L-Glutamine and/or Penicillin-Streptomycin, RPMI 1640, with L-Glutamine, RPMI 1640, without L-Glutamine, Schneider's Insect Medium are suitable for culturing host cells. In addition, any of the media described in Ham et al., Meth. Enz. 58:44 (1979), Barnes et al., Anal. Biochem. 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. No. Re. 30,985 may be used as culture media for the host cells, the entire teachings of all of which are incorporated herein by reference.

[00223] Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as gentamycin drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

[00224] Host cells can also be used to produce portions of intact proteins, for example, antibodies, including Fab fragments or scFv molecules. It is understood that variations on the above procedure are within the scope of the present invention. For example, in certain embodiments it may be desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an antibody. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to an antigen. The molecules expressed from such truncated DNA molecules are also

encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention and the other heavy and light chain are specific for an antigen other than the target antibody, depending on the specificity of the antibody of the invention, by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

[00225] In a suitable system for recombinant expression of a protein, for example, an antibody or antigen-binding portion thereof, a recombinant expression vector encoding the protein, for example, both an antibody heavy chain and an antibody light chain, is introduced into dhfr-CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the protein gene(s) are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the gene(s). The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the protein, for example, the antibody heavy and light chains, and intact protein, for example, an antibody, is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the protein from the culture medium.

[00226] When using recombinant techniques, the protein, for example, antibodies or antigen binding fragments thereof, can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. In one aspect, if the protein is produced intracellularly, as a first step, the particulate debris, either host cells or lysed cells (e.g., resulting from homogenization), can be removed, e.g., by centrifugation or ultrafiltration. Where the protein is secreted into the medium, supernatants from such expression systems can be first concentrated using a commercially available protein concentration filter, e.g., an Amicon™ or Millipore Pellicon™ ultrafiltration unit.

[00227] Some antibodies can be secreted directly from the cell into the surrounding growth media; others are made intracellularly. For antibodies made intracellularly, the first step of a purification process typically involves: lysis of the cell, which can be done by a variety of methods, including mechanical shear, osmotic shock, or enzymatic treatments. Such disruption releases the entire contents of the cell into the homogenate, and in addition produces subcellular fragments that are difficult to remove due to their small size. These are

generally removed by differential centrifugation or by filtration. Where the antibody is secreted, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, e.g., an Amicon™ or Millipore Pellicon™ ultrafiltration unit. Where the antibody is secreted into the medium, the recombinant host cells can also be separated from the cell culture medium, e.g., by tangential flow filtration. Antibodies can be further recovered from the culture medium using antibody purification methods.

[00228] In accordance with the present invention, modulation of the glycosylation profile of the protein or protein composition produced by recombinant cell culture can be achieved by supplementation of the cell culture media with arabinose, such as L-arabinose, D-arabinose, L-altrose, and/or D-altrose. Specific host cell culture conditions can be used with various cultivation methods including, but not limited to, batch, fed-batch, chemostat and perfusion, and with various cell culture equipment including, but not limited to, shake flasks with or without suitable agitation, spinner flasks, stirred bioreactors, airlift bioreactors, membrane bioreactors, reactors with cells retained on a solid support or immobilized/entrapped as in microporous beads, and any other configuration appropriate for optimal growth and productivity of the desired host cell line.

[00229] The upstream process technologies may be used alone or in combination with the downstream process technologies described below.

[00230] Downstream process

[00231] The protein compositions described herein may be purified using downstream process technologies (e.g., purification or concentration), following production using the upstream process technologies of the present invention. For example, once a clarified solution or mixture comprising the protein has been obtained, separation of the protein from process-related impurities, such as the other proteins produced by the host cell, as well as product-related substances, such acidic or basic variants, is performed. In certain embodiments, the initial steps of the purification methods involve the clarification and primary recovery of an antibody from a sample matrix by methods such as centrifugation, depth filtration and/or viral inactivation/reduction. In certain non-limiting embodiments, further separation is performed using cation exchange chromatography, anion exchange chromatography, and/or multi-mode chromatography. In certain embodiments, a combination of one or more different purification techniques, including affinity separation step(s), ion exchange separation step(s), mixed-mode step(s), and/or hydrophobic

interaction separation step(s) can also be employed. Such additional purification steps separate mixtures of proteins on the basis of their charge, degree of hydrophobicity, and/or size. Continuous and recycle chromatography are also applicable to chromatography methods where the protein is collected in the unbound fraction during chromatography or where the protein is first bound to the chromatography resin and subsequently recovered by washing the media with conditions that elute the bound component. Numerous chromatography resins are commercially available for each of these techniques, allowing accurate tailoring of the purification scheme to the particular protein involved. Each of the separation methods allow proteins to either traverse at different rates through a column, achieving a physical separation that increases as they pass further through the column, or to adhere selectively to a separation resin (or medium). The proteins are then differentially eluted using different eluents. In some cases, the protein is separated from impurities when the impurities specifically adhere to the column's resin and the protein does not, i.e., the protein is contained in the effluent, while in other cases the protein will adhere to the column's resin, while the impurities and/or product-related substances are extruded from the column's resin during a wash cycle. Following chromatographic polishing steps the protein compositions of the invention may be further purified using viral filtration. Ultrafiltration and/or diafiltration may be used to further concentrate and formulate the protein.

[00232] The glycosylation profile of the recombinant glycoproteins and glycoprotein compositions prepared by the methods of the invention can be analyzed using methods well known to those skilled in the art, e.g., removal and derivatization of N-glycans followed by NP-HPLC analysis, weak cation exchange chromatography (WCX), capillary isoelectric focusing (cIEF), size-exclusion chromatography, Poros A HPLC Assay, Host cell Protein ELISA, DNA assay, and western blot analysis.

[00233] Techniques for the determination of glycan primary structure, as well as polypeptides comprising glycans of interest, are well known in the art and are described in detail, for example, in Montreuil, "Structure and Biosynthesis of Glycopeptides" In *Polysaccharides in Medicinal Applications*, pp. 273-327, 1996, Eds. Severian Damitriu, Marcel Dekker, NY. It is therefore a routine matter for one of ordinary skill in the art to isolate a population of peptides or polypeptides produced by a cell and determine the structure(s) of the glycans attached thereto. For example, efficient methods are available for (i) the splitting of glycosidic bonds either by chemical cleavage such as hydrolysis, acetolysis, hydrazinolysis, or by nitrous deamination; (ii) complete methylation followed by

hydrolysis or methanolysis and by gas-liquid chromatography and mass spectroscopy of the partially methylated monosaccharides; and (iii) the definition of anomeric linkages between monosaccharides using exoglycosidases, which also provide insight into the primary glycan structure by sequential degradation. Fluorescent labeling and subsequent high performance liquid chromatography (HPLC), e.g., normal phase HPLC (NP-HPLC), mass spectroscopy and nuclear magnetic resonance (NMR) spectrometry, e.g., high field NMR, may also be used to determine glycan primary structure.

[00234] Kits and equipment for carbohydrate analysis are also commercially available. Fluorophore Assisted Carbohydrate Electrophoresis (FACE) is available from Glyko, Inc. (Novato, Calif.). In FACE analysis, glycoconjugates are released from the peptide with either Endo H or N-glycanase (PNGase F) for N-linked glycans, or hydrazine for Ser/Thr linked glycans. The glycan is then labeled at the reducing end with a fluorophore in a non-structure discriminating manner. The fluorophore labeled glycans are then separated in polyacrylamide gels based on the charge/mass ratio of the saccharide as well as the hydrodynamic volume. Images are taken of the gel under UV light and the composition of the glycans is determined by the migration distance as compared with the standards. Oligosaccharides can be sequenced in this manner by analyzing migration shifts due to the sequential removal of saccharides by exoglycosidase digestion.

[00235] C. METHODS OF USE

[00236] The glycoprotein compositions described herein, including, but not limited to, glycoprotein compositions having increased arabinosylation, reduced fucosylation, and/or reduced mannosylation relative to a control composition, may be used to treat any disorder in a subject for which the therapeutic protein (e.g., an antibody) comprised in the composition is appropriate for treating.

[00237] A “disorder” is any condition that would benefit from treatment with the therapeutic protein. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the subject to the disorder in question. In the case of an anti-TNF α antibody, or antigen binding fragment thereof, a therapeutically effective amount of a glycoprotein composition may be administered to treat a disorder in which TNF α activity is detrimental.

[00238] As used herein, the term “subject” is intended to include living organisms, e.g., prokaryotes and eukaryotes. Examples of subjects include mammals, e.g., humans,

dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. In some embodiments, the subject is a human.

[00239] As used herein, the term “treatment” or “treat” refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder, as well as those in which the disorder is to be prevented.

[00240] In one embodiment, the invention provides a method of administering a glycoprotein composition comprising, for example, an arabinosylated antibody or antigen binding fragment thereof to a subject such that the activity of the target containing the antigen is inhibited or a disorder in which such target activity is detrimental is treated. In one embodiment, the TNF α is human TNF α and the subject is a human subject.

[00241] The glycoprotein compositions can be administered by a variety of methods known in the art. Exemplary routes/modes of administration include subcutaneous injection, intravenous injection or infusion. In certain aspects, a glycoprotein composition may be orally administered. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

[00242] Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. In certain embodiments it is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit comprising a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[00243] An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of a glycoprotein composition is 0.01-20 mg/kg, or 1-10 mg/kg, or 0.3-1 mg/kg.

[00244] It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[00245] D. PHARMACEUTICAL COMPOSITIONS

[00246] The present invention further provides preparations and formulations comprising a glycoprotein composition with a glycosylation profile described herein, for example an arabinosylated glycoprotein composition, optionally with a decreased fucosylation level and/or decreased mannosylation level relative to a control composition. It should be understood that any of the glycoprotein compositions may be formulated or prepared as described below. In certain embodiments, the glycoprotein composition comprises an antibody or antigen-binding portion thereof, such as an anti-TNF α antibody or antigen-binding portion thereof.

[00247] In certain embodiments, the glycoprotein compositions may be formulated with a pharmaceutically acceptable carrier as pharmaceutical (therapeutic) compositions, and may be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. The term “pharmaceutically acceptable carrier” means one or more non-toxic materials that do not interfere with the effectiveness of the biological activity of the active ingredients. Such preparations may routinely contain salts, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. Such pharmaceutically acceptable preparations may also routinely contain compatible solid or liquid fillers, diluents or encapsulating substances which are suitable for administration into a human. The term “carrier” denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the glycoprotein composition, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

[00248] The glycoprotein compositions are present in a form known in the art and acceptable for therapeutic uses. In certain embodiments, a formulation comprising a glycoprotein composition is a liquid formulation. In another embodiment, a formulation

comprising a glycoprotein composition is a lyophilized formulation. In a further embodiment, a formulation comprising a glycoprotein composition is a reconstituted liquid formulation. In certain embodiments, a formulation comprising a glycoprotein composition is a stable liquid formulation. In certain embodiments, a liquid formulation comprising a glycoprotein composition is an aqueous formulation. In another embodiment, the liquid formulation is non-aqueous. In a specific embodiment, a liquid formulation comprising a glycoprotein composition is an aqueous formulation wherein the aqueous carrier is distilled water.

[00249] In certain embodiments, the formulations comprise a glycoprotein composition in a concentration resulting in a w/v appropriate for a desired dose. The glycoprotein composition may be present in the formulation at a concentration of about 1 mg/mL to about 500 mg/mL, e.g., at a concentration of at least 1 mg/mL, at least 5 mg/mL, at least 10 mg/mL, at least 15 mg/mL, at least 20 mg/mL, at least 25 mg/mL, at least 30 mg/mL, at least 35 mg/mL, at least 40 mg/mL, at least 45 mg/mL, at least 50 mg/mL, at least 55 mg/mL, at least 60 mg/mL, at least 65 mg/mL, at least 70 mg/mL, at least 75 mg/mL, at least 80 mg/mL, at least 85 mg/mL, at least 90 mg/mL, at least 95 mg/mL, at least 100 mg/mL, at least 105 mg/mL, at least 110 mg/mL, at least 115 mg/mL, at least 120 mg/mL, at least 125 mg/mL, at least 130 mg/mL, at least 135 mg/mL, at least 140 mg/mL, at least 150 mg/mL, at least 200 mg/mL, at least 250 mg/mL, or at least 300 mg/mL.

[00250] In some embodiments, a formulation comprises at least about 50 mg/mL, at least about 100 mg/mL, at least about 125 mg/mL, at least 130 mg/mL, or at least about 150 mg/mL of the glycoprotein composition.

[00251] In certain embodiments, the concentration of a glycoprotein in the glycoprotein composition that is included in the formulation is between about 1 mg/mL and about 25 mg/mL, between about 1 mg/mL and about 200 mg/mL, between about 25 mg/mL and about 200 mg/mL, between about 50 mg/mL and about 200 mg/mL, between about 75 mg/mL and about 200 mg/mL, between about 100 mg/mL and about 200 mg/mL, between about 125 mg/mL and about 200 mg/mL, between about 150 mg/mL and about 200 mg/mL, between about 25 mg/mL and about 150 mg/mL, between about 50 mg/mL and about 150 mg/mL, between about 75 mg/mL and about 150 mg/mL, between about 100 mg/mL and about 150 mg/mL, between about 125 mg/mL and about 150 mg/mL, between about 25 mg/mL and about 125 mg/mL, between about 50 mg/mL and about 125 mg/mL, between about 75 mg/mL and about 125 mg/mL, between about 100 mg/mL and about 125 mg/mL,

between about 25 mg/mL and about 100 mg/mL, between about 50 mg/mL and about 100 mg/mL, between about 75 mg/mL and about 100 mg/mL, between about 25 mg/mL and about 75 mg/mL, between about 50 mg/mL and about 75 mg/mL, or between about 25 mg/mL and about 50 mg/mL.

[00252] In a specific embodiment, a formulation comprises between about 40 mg/mL and about 110 mg/mL or between about 50 mg/mL and about 210 mg/mL of a glycoprotein composition.

[00253] The formulations comprising a glycoprotein composition may further comprise one or more active compounds as necessary for the particular indication being treated, typically those with complementary activities that do not adversely affect each other. Such additional active compounds are suitably present in combination in amounts that are effective for the purpose intended.

[00254] The formulations comprising a glycoprotein composition may be prepared for storage by mixing a glycoprotein having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers, including, but not limited to buffering agents, saccharides, salts, surfactants, solubilizers, polyols, diluents, binders, stabilizers, salts, lipophilic solvents, amino acids, chelators, preservatives, or the like (Goodman and Gilman's *The Pharmacological Basis of Therapeutics*, 12th edition, L. Brunton, et al. and Remington's *Pharmaceutical Sciences*, 16th edition, Osol, A. Ed. (1999)), in the form of lyophilized formulations or aqueous solutions at a desired final concentration. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as histidine, phosphate, citrate, glycine, acetate and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including trehalose, glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal

complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN, polysorbate 80, PLURONICS® or polyethylene glycol (PEG).

[00255] The buffering agent may be histidine, citrate, phosphate, glycine, or acetate. The saccharide excipient may be trehalose, sucrose, mannitol, maltose or raffinose. The surfactant may be polysorbate 20, polysorbate 40, polysorbate 80, or Pluronic F68. The salt may be NaCl, KCl, MgCl₂, or CaCl₂.

[00256] The formulations comprising a glycoprotein composition may include a buffering or pH adjusting agent to provide improved pH control. Such a formulation may have a pH of between about 3.0 and about 9.0, between about 4.0 and about 8.0, between about 5.0 and about 8.0, between about 5.0 and about 7.0, between about 5.0 and about 6.5, between about 5.5 and about 8.0, between about 5.5 and about 7.0, or between about 5.5 and about 6.5. In a further embodiment, such a formulation has a pH of about 3.0, about 3.5, about 4.0, about 4.5, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.5, about 8.0, about 8.5, or about 9.0. In a specific embodiment, a formulation has a pH of about 6.0. One of skill in the art understands that the pH of a formulation generally should not be equal to the isoelectric point of the particular a glycoprotein (e.g., antibody) to be used in the formulation.

[00257] Typically, the buffering agent is a salt prepared from an organic or inorganic acid or base. Representative buffering agents include, but are not limited to, organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. In addition, amino acid components can also function in a buffering capacity. Representative amino acid components which may be utilized in the formulations as buffering agents include, but are not limited to, glycine and histidine. In certain embodiments, the buffering agent is chosen from histidine, citrate, phosphate, glycine, and acetate. In a specific embodiment, the buffering agent is histidine. In another specific embodiment, the buffering agent is citrate. In yet another specific embodiment, the buffering agent is glycine. The purity of the buffering agent should be at least 98%, or at least 99%, or at least 99.5%. As used herein, the term “purity” in the context of histidine and glycine refers to chemical purity of histidine or glycine as understood in the art, e.g., as described in The Merck Index, 13th ed., O’Neil et al. ed. (Merck & Co., 2001).

[00258] Buffering agents are typically used at concentrations between about 1 mM and about 200 mM or any range or value therein, depending on the desired ionic strength and the buffering capacity required. The usual concentrations of conventional buffering agents employed in parenteral formulations can be found in: *Pharmaceutical Dosage Form: Parenteral Medications*, Volume 1, 2nd Edition, Chapter 5, p. 194, De Luca and Boylan, "Formulation of Small Volume Parenterals", Table 5: Commonly used additives in Parenteral Products. In certain embodiments, the buffering agent is at a concentration of at least about 1 mM, or of at least about 5 mM, or of at least about 10 mM, or of at least about 15 mM, or of at least about 20 mM, or of at least about 25 mM, or of at least about 30 mM, or of at least about 35 mM, or of at least about 40 mM, or of at least about 45 mM, or of at least about 50 mM, or of at least about 60 mM, or of at least about 70 mM, or of at least about 80 mM, or of at least about 90 mM, or of at least about 100 mM. In certain embodiments, the buffering agent is at a concentration of about 1 mM, or of about 5 mM, or of about 10 mM, or of about 15 mM, or of about 20 mM, or of about 25 mM, or of about 30 mM, or of about 35 mM, or of about 40 mM, or of about 45 mM, or of about 50 mM, or of about 60 mM, or of about 70 mM, or of about 80 mM, or of about 90 mM, or of about 100 mM. In a specific embodiment, the buffering agent is at a concentration of between about 5 mM and about 50 mM. In another specific embodiment, the buffering agent is at a concentration of between 5 mM and 20 mM.

[00259] In certain embodiments, the formulation comprising a glycoprotein composition comprises histidine as a buffering agent. In certain embodiments the histidine is present in the formulation at a concentration of at least about 1 mM, at least about 5 mM, at least about 10 mM, at least about 20 mM, at least about 30 mM, at least about 40 mM, at least about 50 mM, at least about 75 mM, at least about 100 mM, at least about 150 mM, or at least about 200 mM histidine. In another embodiment, a formulation comprises between about 1 mM and about 200 mM, between about 1 mM and about 150 mM, between about 1 mM and about 100 mM, between about 1 mM and about 75 mM, between about 10 mM and about 200 mM, between about 10 mM and about 150 mM, between about 10 mM and about 100 mM, between about 10 mM and about 75 mM, between about 10 mM and about 50 mM, between about 10 mM and about 40 mM, between about 10 mM and about 30 mM, between about 20 mM and about 75 mM, between about 20 mM and about 50 mM, between about 20 mM and about 40 mM, or between about 20 mM and about 30 mM histidine. In a further embodiment, the formulation comprises about 1 mM, about 5 mM,

about 10 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, about 100 mM, about 150 mM, or about 200 mM histidine. In a specific embodiment, a formulation may comprise about 10 mM, about 25 mM, or no histidine.

[00260] The formulations comprising a glycoprotein composition may comprise a carbohydrate excipient. Carbohydrate excipients can act, e.g., as viscosity enhancing agents, stabilizers, bulking agents, solubilizing agents, and/or the like. Carbohydrate excipients are generally present at between about 1% to about 99% by weight or volume, e.g., between about 0.1% to about 20%, between about 0.1% to about 15%, between about 0.1% to about 5%, between about 1% to about 20%, between about 5% to about 15%, between about 8% to about 10%, between about 10% and about 15%, between about 15% and about 20%, between 0.1% to 20%, between 5% to 15%, between 8% to 10%, between 10% and 15%, between 15% and 20%, between about 0.1% to about 5%, between about 5% to about 10%, or between about 15% to about 20%. In still other specific embodiments, the carbohydrate excipient is present at 1%, or at 1.5%, or at 2%, or at 2.5%, or at 3%, or at 4%, or at 5%, or at 10%, or at 15%, or at 20%.

[00261] Carbohydrate excipients suitable for use in the formulations include, but are not limited to, monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol) and the like. In certain embodiments, the carbohydrate excipients for use in the present invention are chosen from sucrose, trehalose, lactose, mannitol, and raffinose. In a specific embodiment, the carbohydrate excipient is trehalose. In another specific embodiment, the carbohydrate excipient is mannitol. In yet another specific embodiment, the carbohydrate excipient is sucrose. In still another specific embodiment, the carbohydrate excipient is raffinose. The purity of the carbohydrate excipient should be at least 98%, or at least 99%, or at least 99.5%.

[00262] In a specific embodiment, the formulations comprising a glycoprotein composition may comprise trehalose. In certain embodiments, a formulation comprises at least about 1%, at least about 2%, at least about 4%, at least about 8%, at least about 20%, at least about 30%, or at least about 40% trehalose. In another embodiment, a formulation comprises between about 1% and about 40%, between about 1% and about 30%, between

about 1% and about 20%, between about 2% and about 40%, between about 2% and about 30%, between about 2% and about 20%, between about 4% and about 40%, between about 4% and about 30%, or between about 4% and about 20% trehalose. In a further embodiment, a formulation comprises about 1%, about 2%, about 4%, about 6%, about 8%, about 15%, about 20%, about 30%, or about 40% trehalose. In a specific embodiment, a formulation comprises about 4%, about 6% or about 15% trehalose.

[00263] In certain embodiments, a formulation comprising a glycoprotein composition comprises an excipient. In a specific embodiment, a formulation comprises at least one excipient chosen from: sugar, salt, surfactant, amino acid, polyol, chelating agent, emulsifier and preservative. In certain embodiments, a formulation comprises a salt, e.g., a salt selected from: NaCl, KCl, CaCl₂, and MgCl₂. In a specific embodiment, the formulation comprises NaCl.

[00264] A formulation comprising a glycoprotein composition may comprise at least about 10 mM, at least about 25 mM, at least about 50 mM, at least about 75 mM, at least about 80 mM, at least about 100 mM, at least about 125 mM, at least about 150 mM, at least about 175 mM, at least about 200 mM, or at least about 300 mM sodium chloride (NaCl). In a further embodiment, the formulation may comprise between about 10 mM and about 300 mM, between about 10 mM and about 200 mM, between about 10 mM and about 175 mM, between about 10 mM and about 150 mM, between about 25 mM and about 300 mM, between about 25 mM and about 200 mM, between about 25 mM and about 175 mM, between about 25 mM and about 150 mM, between about 50 mM and about 300 mM, between about 50 mM and about 200 mM, between about 50 mM and about 175 mM, between about 50 mM and about 150 mM, between about 75 mM and about 300 mM, between about 75 mM and about 200 mM, between about 75 mM and about 175 mM, between about 75 mM and about 150 mM, between about 100 mM and about 300 mM, between about 100 mM and about 200 mM, between about 100 mM and about 175 mM, or between about 100 mM and about 150 mM sodium chloride. In a further embodiment, the formulation may comprise about 10 mM, about 25 mM, about 50 mM, about 75 mM, about 80 mM, about 100 mM, about 125 mM, about 150 mM, about 175 mM, about 200 mM, or about 300 mM sodium chloride.

[00265] A formulation comprising a glycoprotein composition may also comprise an amino acid, e.g., lysine, arginine, glycine, histidine or an amino acid salt. The formulation may comprise at least about 1 mM, at least about 10 mM, at least about 25 mM, at least

about 50 mM, at least about 100 mM, at least about 150 mM, at least about 200 mM, at least about 250 mM, at least about 300 mM, at least about 350 mM, or at least about 400 mM of an amino acid. In another embodiment, the formulation may comprise between about 1 mM and about 100 mM, between about 10 mM and about 150 mM, between about 25 mM and about 250 mM, between about 25 mM and about 300 mM, between about 25 mM and about 350 mM, between about 25 mM and about 400 mM, between about 50 mM and about 250 mM, between about 50 mM and about 300 mM, between about 50 mM and about 350 mM, between about 50 mM and about 400 mM, between about 100 mM and about 250 mM, between about 100 mM and about 300 mM, between about 100 mM and about 400 mM, between about 150 mM and about 250 mM, between about 150 mM and about 300 mM, or between about 150 mM and about 400 mM of an amino acid. In a further embodiment, a formulation comprises about 1 mM, 1.6 mM, 25 mM, about 50 mM, about 100 mM, about 150 mM, about 200 mM, about 250 mM, about 300 mM, about 350 mM, or about 400 mM of an amino acid.

[00266] The formulations comprising a glycoprotein composition may further comprise a surfactant. The term “surfactant” as used herein refers to organic substances having amphipathic structures; namely, they are composed of groups of opposing solubility tendencies, typically an oil-soluble hydrocarbon chain and a water-soluble ionic group. Surfactants can be classified, depending on the charge of the surface-active moiety, into anionic, cationic, and nonionic surfactants. Surfactants are often used as wetting, emulsifying, solubilizing, and dispersing agents for various pharmaceutical compositions and preparations of biological materials. Pharmaceutically acceptable surfactants like polysorbates (e.g., polysorbates 20 or 80); polyoxamers (e.g., poloxamer 188); Triton; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (e.g., lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUA® series (Mona Industries, Inc., Paterson, N.J.), polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (e.g., PLURONICS® PF68, etc.), can optionally be added to the formulations to reduce aggregation. In certain embodiments, a formulation comprises Polysorbate 20, Polysorbate 40, Polysorbate 60, or Polysorbate 80. Surfactants are

particularly useful if a pump or plastic container is used to administer the formulation. The presence of a pharmaceutically acceptable surfactant mitigates the propensity for the protein to aggregate. The formulations may comprise a polysorbate which is at a concentration ranging from between about 0.001% to about 1%, or about 0.001% to about 0.1%, or about 0.01% to about 0.1%. In other specific embodiments, the formulations comprise a polysorbate which is at a concentration of 0.001%, or 0.002%, or 0.003%, or 0.004%, or 0.005%, or 0.006%, or 0.007%, or 0.008%, or 0.009%, or 0.01%, or 0.015%, or 0.02%.

[00267] The formulations comprising a glycoprotein composition may optionally further comprise other common excipients and/or additives including, but not limited to, diluents, binders, stabilizers, lipophilic solvents, preservatives, adjuvants, or the like. Pharmaceutically acceptable excipients and/or additives may be used in the formulations of the invention. Commonly used excipients/additives, such as pharmaceutically acceptable chelators (for example, but not limited to, EDTA, DTPA or EGTA) can optionally be added to the formulations to reduce aggregation. These additives are particularly useful if a pump or plastic container is used to administer the formulation.

[00268] Preservatives, such as phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, phenylmercuric nitrite, phenoxyethanol, formaldehyde, chlorobutanol, magnesium chloride (for example, but not limited to, hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof can optionally be added to the formulations at any suitable concentration such as between about 0.001% to about 5%, or any range or value therein. The concentration of preservative used in the formulations is a concentration sufficient to yield a microbial effect. Such concentrations are dependent on the preservative selected and are readily determined by the skilled artisan.

[00269] Other contemplated excipients/additives, which may be utilized in the formulations include, for example, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, lipids such as phospholipids or fatty acids, steroids such as cholesterol, protein excipients such as serum albumin (human serum albumin (HSA), recombinant human albumin (rHA)), gelatin, casein, salt-forming counterions such as sodium and the like. These and additional known pharmaceutical excipients and/or additives suitable for use in the formulations are known in the art, e.g., as listed in "Remington: The Science & Practice of Pharmacy", 21st ed., Lippincott Williams &

Wilkins, (2005), and in the "Physician's Desk Reference", 60th ed., Medical Economics, Montvale, N.J. (2005). Pharmaceutically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the glycoprotein of the glycoprotein composition, as well known those in the art or as described herein.

[00270] It will be understood by one skilled in the art that the formulations comprising a glycoprotein composition may be isotonic with human blood, wherein the formulations have essentially the same osmotic pressure as human blood. Such isotonic formulations will generally have an osmotic pressure from about 250 mOSm to about 350 mOSm. Isotonicity can be measured by, for example, using a vapor pressure or ice-freezing type osmometer. Tonicity of a formulation is adjusted by the use of tonicity modifiers. "Tonicity modifiers" are those pharmaceutically acceptable inert substances that can be added to the formulation to provide an isotonicity of the formulation. Tonicity modifiers suitable for this invention include, but are not limited to, saccharides, salts and amino acids.

[00271] In certain embodiments, the formulations comprising a glycoprotein composition have an osmotic pressure from about 100 mOSm to about 1200 mOSm, or from about 200 mOSm to about 1000 mOSm, or from about 200 mOSm to about 800 mOSm, or from about 200 mOSm to about 600 mOSm, or from about 250 mOSm to about 500 mOSm, or from about 250 mOSm to about 400 mOSm, or from about 250 mOSm to about 350 mOSm.

[00272] The concentration of any one component or any combination of various components, of the formulations comprising a glycoprotein composition is adjusted to achieve the desired tonicity of the final formulation. For example, the ratio of the carbohydrate excipient to glycoprotein may be adjusted according to methods known in the art (e.g., U.S. Pat. No. 6,685,940). In certain embodiments, the molar ratio of the carbohydrate excipient to glycoprotein may be from about 100 moles to about 1000 moles of carbohydrate excipient to about 1 mole of glycoprotein, or from about 200 moles to about 6000 moles of carbohydrate excipient to about 1 mole of glycoprotein, or from about 100 moles to about 510 moles of carbohydrate excipient to about 1 mole of glycoprotein, or from about 100 moles to about 600 moles of carbohydrate excipient to about 1 mole of glycoprotein.

[00273] The desired isotonicity of the final formulation may also be achieved by adjusting the salt concentration of the formulations. Pharmaceutically acceptable salts and those suitable as tonicity modifiers include, but are not limited to, sodium chloride, sodium

succinate, sodium sulfate, potassium chloride, magnesium chloride, magnesium sulfate, and calcium chloride. In some embodiments, formulations comprise NaCl, MgCl₂, and/or CaCl₂. In certain embodiments, concentration of NaCl is between about 75 mM and about 150 mM. In another embodiment, concentration of MgCl₂ is between about 1 mM and about 100 mM. Pharmaceutically acceptable amino acids including those suitable as tonicity modifiers include, but are not limited to, proline, alanine, L-arginine, asparagine, L-aspartic acid, glycine, serine, lysine, and histidine.

[00274] In certain embodiments the formulations comprising a glycoprotein composition are pyrogen-free formulations which are substantially free of endotoxins and/or related pyrogenic substances. Endotoxins include toxins that are confined inside a microorganism and are released only when the microorganisms are broken down or die. Pyrogenic substances also include fever-inducing, thermostable substances from the outer membrane of bacteria and other microorganisms. Both of these substances can cause fever, hypotension and shock if administered to humans. Due to the potential harmful effects, even low amounts of endotoxins must be removed from intravenously administered pharmaceutical drug solutions. The Food & Drug Administration (“FDA”) has set an upper limit of 5 endotoxin units (EU) per dose per kilogram body weight in a single one hour period for intravenous drug applications (The United States Pharmacopeial Convention, Pharmacopeial Forum 26 (1):223 (2000)). When therapeutic proteins are administered in amounts of several hundred or thousand milligrams per kilogram body weight, as can be the case with proteins of interest (e.g., antibodies), even trace amounts of harmful and dangerous endotoxin must be removed. In some embodiments, the endotoxin and pyrogen levels in the composition are less than 10 EU/mg, or less than 5 EU/mg, or less than 1 EU/mg, or less than 0.1 EU/mg, or less than 0.01 EU/mg, or less than 0.001 EU/mg.

[00275] When used for in vivo administration, the formulations comprising a glycoprotein composition should be sterile. The formulations may be sterilized by various sterilization methods, including sterile filtration, radiation, etc. In certain embodiments, formulation is filter-sterilized with a presterilized 0.22-micron filter. Sterile compositions for injection can be formulated according to conventional pharmaceutical practice as described in “Remington: The Science & Practice of Pharmacy”, 21st ed., Lippincott Williams & Wilkins, (2005). Formulations comprising proteins of interest (e.g., antibody or other glycoprotein), such as those disclosed herein, ordinarily will be stored in lyophilized form or in solution. It is contemplated that sterile compositions comprising proteins of

interest (e.g., antibody or other glycoprotein) are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having an adapter that allows retrieval of the formulation, such as a stopper pierceable by a hypodermic injection needle. In certain embodiments, a composition is provided as a pre-filled syringe.

[00276] In certain embodiments, a formulation comprising a glycoprotein composition is a lyophilized formulation. The term “lyophilized” or “freeze-dried” includes a state of a substance that has been subjected to a drying procedure such as lyophilization, where at least 50% of moisture has been removed.

[00277] The phrase “bulking agent” includes a compound that is pharmaceutically acceptable and that adds bulk to a lyo cake. Bulking agents known to the art include, for example, carbohydrates, including simple sugars such as dextrose, ribose, fructose and the like, alcohol sugars such as mannitol, inositol and sorbitol, disaccharides including trehalose, sucrose and lactose, naturally occurring polymers such as starch, dextrans, chitosan, hyaluronate, proteins (e.g., gelatin and serum albumin), glycogen, and synthetic monomers and polymers.

[00278] A “lyoprotectant” is a molecule which, when combined with a glycoprotein composition, significantly prevents or reduces chemical and/or physical instability of the protein upon lyophilization and subsequent storage. Lyoprotectants include, but are not limited to, sugars and their corresponding sugar alcohols; an amino acid or salt thereof such as monosodium glutamate or histidine; a methylamine such as betaine; a lyotropic salt such as magnesium sulfate; a polyol such as trihydric or higher molecular weight sugar alcohols, e.g., glycerin, dextran, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol; propylene glycol; polyethylene glycol; PLURONICS®; and combinations thereof. Additional examples of lyoprotectants include, but are not limited to, glycerin and gelatin, and the sugars mellibiose, melezitose, raffinose, mannotriose and stachyose. Examples of reducing sugars include, but are not limited to, glucose, maltose, lactose, maltulose, iso-maltulose and lactulose. Examples of non-reducing sugars include, but are not limited to, non-reducing glycosides of polyhydroxy compounds selected from sugar alcohols and other straight chain polyalcohols. Examples of sugar alcohols include, but are not limited to, monoglycosides, compounds obtained by reduction of disaccharides such as lactose, maltose, lactulose and maltulose. The glycosidic side group can be either glucosidic or galactosidic. Additional examples of sugar alcohols include, but are not limited to, glucitol,

maltitol, lactitol and iso-maltulose. In some embodiments, trehalose or sucrose is used as a lyoprotectant.

[00279] The lyoprotectant is added to the pre-lyophilized formulation in a “lyoprotecting amount” which means that, following lyophilization of the protein in the presence of the lyoprotecting amount of the lyoprotectant, the protein essentially retains its physical and chemical stability and integrity upon lyophilization and storage.

[00280] In certain embodiments, the molar ratio of a lyoprotectant (e.g., trehalose) and a glycoprotein in the formulation is at least about 10, at least about 50, at least about 100, at least about 200, or at least about 300. In another embodiment, the molar ratio of a lyoprotectant (e.g., trehalose) and a glycoprotein in the formulation is about 1, is about 2, is about 5, is about 10, about 50, about 100, about 200, or about 300.

[00281] A “reconstituted” formulation is one which has been prepared by dissolving a lyophilized glycoprotein composition in a diluent such that the glycoprotein molecules are dispersed in the reconstituted formulation. The reconstituted formulation is suitable for administration (e.g., parenteral administration) to a patient to be treated with the glycoprotein composition and, in certain embodiments of the invention, may be one which is suitable for intravenous administration.

[00282] The “diluent” of interest herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a liquid formulation, such as a formulation reconstituted after lyophilization. In some embodiments, diluents include, but are not limited to, sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (e.g., phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution. In an alternative embodiment, diluents can include aqueous solutions of salts and/or buffers.

[00283] In certain embodiments, a formulation comprising a glycoprotein composition is a lyophilized formulation, wherein at least about 90%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% of the glycoprotein molecules may be recovered from a vial upon shaking the vial for 4 hours at a speed of 400 shakes per minute wherein the vial is filled to half of its volume with the formulation. In another embodiment, a formulation is a lyophilized formulation, wherein at least about 90%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% of the glycoprotein molecules may be recovered from a vial upon subjecting the formulation to three freeze/thaw cycles wherein the vial is filled to half of its volume with the formulation. In a

further embodiment, a formulation is a lyophilized formulation, wherein at least about 90%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% of the glycoprotein molecules may be recovered by reconstituting a lyophilized cake generated from the formulation.

[00284] In certain embodiments, a reconstituted liquid formulation may comprise a glycoprotein composition at the same concentration as the pre-lyophilized liquid formulation.

[00285] In another embodiment, a reconstituted liquid formulation may comprise a glycoprotein composition at a higher concentration than the pre-lyophilized liquid formulation, e.g., about 2 fold, about 3 fold, about 4 fold, about 5 fold, about 6 fold, about 7 fold, about 8 fold, about 9 fold, or about 10 fold higher concentration of a glycoprotein composition than the pre-lyophilized liquid formulation.

[00286] In yet another embodiment, a reconstituted liquid formulation may comprise a glycoprotein composition at a lower concentration than the pre-lyophilized liquid formulation, e.g., about 2 fold, about 3 fold, about 4 fold, about 5 fold, about 6 fold, about 7 fold, about 8 fold, about 9 fold or about 10 fold lower concentration of a glycoprotein composition than the pre-lyophilized liquid formulation.

[00287] The pharmaceutical formulations comprising a glycoprotein composition are typically stable formulations, e.g., stable at room temperature.

[00288] The terms “stability” and “stable” as used herein in the context of a formulation comprising a glycoprotein composition refer to the resistance of the glycoprotein in the formulation to aggregation, degradation or fragmentation under given manufacture, preparation, transportation and storage conditions. The “stable” formulations retain biological activity under given manufacture, preparation, transportation and storage conditions. The stability of the glycoprotein composition can be assessed by degrees of aggregation, degradation or fragmentation, as measured by HPSEC, static light scattering (SLS), Fourier Transform Infrared Spectroscopy (FTIR), circular dichroism (CD), urea unfolding techniques, intrinsic tryptophan fluorescence, differential scanning calorimetry, and/or ANS binding techniques, compared to a reference formulation. For example, a reference formulation may be a reference standard frozen at -70° C. consisting of 10 mg/mL of a glycoprotein in PBS.

[00289] Therapeutic formulations comprising a glycoprotein composition may be formulated for a particular dosage. Dosage regimens may be adjusted to provide the

optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms are dictated by and directly dependent on (a) the unique characteristics of the glycoprotein, and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such a glycoprotein for the treatment of sensitivity in individuals.

[00290] Therapeutic compositions comprising a glycosylation profile described herein, can be formulated for particular routes of administration, such as oral, nasal, pulmonary, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. By way of example, in certain embodiments, glycoprotein compositions are formulated for intravenous administration. In certain other embodiments, glycoprotein compositions are formulated for local delivery to the cardiovascular system, for example, via catheter, stent, wire, intramyocardial delivery, intrapericardial delivery, or intraendocardial delivery.

[00291] Formulations comprising a glycoprotein composition, which are suitable for topical or transdermal administration include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The glycoprotein may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required (U.S. Pat. Nos. 7,378,110; 7,258,873; 7,135,180; 7,923,029; and US Publication No. 20040042972).

[00292] The phrases “parenteral administration” and “administered parenterally” as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

[00293] Actual dosage levels of the glycoprotein in the pharmaceutical compositions comprising a glycosylation profile described herein, may be varied so as to obtain an amount of the glycoprotein which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[00294] In certain embodiments, glycoprotein compositions can be formulated to ensure proper distribution in vivo. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds can cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Pat. Nos. 4,522,811; 5,374,548; 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, e.g., V. V. Ranade (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (see, e.g., U.S. Pat. No. 5,416,016); mannosides (Umezawa et al., (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (P. G. Bloeman et al. (1995) *FEBS Lett.* 357:140; M. Owais et al. (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant Protein A receptor (Briscoe et al. (1995) *Am. J. Physiol.* 1233:134), different species of which may comprise the formulations, as well as components of the invented molecules; p 120 (Schreier et al. (1994) *J. Biol. Chem.* 269:9090); see also K. Keinänen; M. L. Laukkanen (1994) *FEBS Lett.* 346:123; J. J. Killian; I. J. Fidler (1994) *Immunomethods* 4:273. In certain embodiments, the glycoproteins are formulated in liposomes; in another embodiment, the liposomes

include a targeting moiety. In another embodiment, the glycoproteins in the liposomes are delivered by bolus injection to a site proximal to the desired area. When administered in this manner, the composition must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and may be preserved against the contaminating action of microorganisms such as bacteria and fungi. Additionally or alternatively, the proteins with modulated glycosylation profiles (e.g., antibodies) may be delivered locally to the brain to mitigate the risk that the blood brain barrier slows effective delivery.

[00295] In certain embodiments, the glycoprotein compositions may be administered with medical devices known in the art. For example, in certain embodiments, the glycoprotein composition is administered locally via a catheter, stent, wire, or the like. For example, in certain embodiments, a therapeutic composition can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Pat. Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; 4,596,556. Examples of well-known implants and modules include: U.S. Pat. No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Pat. No. 4,486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Pat. No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Pat. No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Pat. No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Pat. No. 4,475,196, which discloses an osmotic drug delivery system. Many other such implants, delivery systems, and modules are known to those skilled in the art.

[00296] The efficient dosages and the dosage regimens for the compositions comprising a glycosylation profile described herein depend on the disease or condition to be treated and can be determined by the persons skilled in the art. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

[00297] E. KITS AND ARTICLES OF MANUFACTURE

[00298] Also within the scope of the present invention are kits comprising the compositions comprising a glycosylation profile described herein, for example an

arabinosylated glycoprotein such as an antibody or antigen-binding portion thereof, optionally with a decreased fucosylation level or amount and/or an decreased mannosylation level or amount and instructions for use. The term “kit” as used herein refers to a packaged product comprising components with which to administer the glycoprotein composition for treatment of a disease or disorder. The kit may comprise a box or container that holds the components of the kit. The box or container is affixed with a label or a Food and Drug Administration approved protocol. The box or container holds components which may be contained within plastic, polyethylene, polypropylene, ethylene, or propylene vessels. The vessels can be capped-tubes or bottles. The kit can also include instructions for administering a glycoprotein composition.

[00299] The kit can further contain one more additional reagents, such as an immunosuppressive reagent, a cytotoxic agent or a radiotoxic agent or one or more additional proteins of interest (e.g., an antibody having a complementary activity which binds to an epitope in the TNF α antigen distinct from a first anti-TNF α antibody). Kits typically include a label indicating the intended use of the contents of the kit. The term label includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit.

[00300] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with a liquid formulation or lyophilized formulation of a glycoprotein composition. In certain embodiments, a container filled with a liquid formulation is a pre-filled syringe. In a specific embodiment, the formulations are formulated in single dose vials as a sterile liquid. For example, the formulations may be supplied in 3 cc USP Type I borosilicate amber vials (West Pharmaceutical Services--Part No. 6800-0675) with a target volume of 1.2 mL. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00301] In certain embodiments, a container filled with a liquid formulation is a pre-filled syringe. Any pre-filled syringe known to one of skill in the art may be used in combination with a liquid formulation of the invention. Pre-filled syringes that may be used are described in, for example, but not limited to, PCT Publications WO05032627, WO08094984, WO9945985, WO03077976, U.S. Pat. No. 6,792,743, U.S. Pat. No. 5,607,400, U.S. Pat. No. 5,893,842, U.S. Pat. No. 7,081,107, U.S. Pat. No. 7,041,087, U.S.

Pat. No. 5,989,227, U.S. Pat. No. 6,807,797, U.S. Pat. No. 6,142,976, U.S. Pat. No. 5,899,889, U.S. Pat. No. 7,699,811, U.S. Pat. No. 7,540,382, U.S. Pat. No. 7,998,120, U.S. Pat. No. 7,645,267, and US Patent Publication No. US20050075611. Pre-filled syringes may be made of various materials. In certain embodiments a pre-filled syringe is a glass syringe. In another embodiment a pre-filled syringe is a plastic syringe. One of skill in the art understands that the nature and/or quality of the materials used for manufacturing the syringe may influence the stability of a protein formulation stored in the syringe. For example, it is understood that silicon based lubricants deposited on the inside surface of the syringe chamber may affect particle formation in the protein formulation. In certain embodiments, a pre-filled syringe comprises a silicone based lubricant. In certain embodiments, a pre-filled syringe comprises baked on silicone. In another embodiment, a pre-filled syringe is free from silicone based lubricants. One of skill in the art also understands that small amounts of contaminating elements leaching into the formulation from the syringe barrel, syringe tip cap, plunger or stopper may also influence stability of the formulation. For example, it is understood that tungsten introduced during the manufacturing process may adversely affect formulation stability. In certain embodiments, a pre-filled syringe may comprise tungsten at a level above 500 ppb. In another embodiment, a pre-filled syringe is a low tungsten syringe. In another embodiment, a pre-filled syringe may comprise tungsten at a level between about 500 ppb and about 10 ppb, between about 400 ppb and about 10 ppb, between about 300 ppb and about 10 ppb, between about 200 ppb and about 10 ppb, between about 100 ppb and about 10 ppb, between about 50 ppb and about 10 ppb, between about 25 ppb and about 10 ppb.

[00302] In certain embodiments, kits comprising an arbinosylated glycoprotein composition, optionally having a decreased fucosylation level or amount and/or a decreased mannosylation level or amount, are also provided that are useful for various purposes, e.g., research and diagnostic including for purification or immunoprecipitation from cells or detection in vitro or in vivo. For isolation and purification, the kit may contain an antibody coupled to beads (e.g., sepharose beads). Kits may be provided which contain the antibodies for detection and quantitation of glycoprotein molecules in vitro, e.g., in an ELISA or a Western blot. As with the article of manufacture, the kit comprises a container and a label or package insert on or associated with the container. The container holds a composition comprising at least one glycoprotein composition. Additional containers may be included that contain, e.g., diluents and buffers, control compositions. The label or

package insert may provide a description of the composition as well as instructions for the intended *in vitro* or diagnostic use.

[00303] The present invention also encompasses a finished packaged and labeled pharmaceutical product. This article of manufacture includes the appropriate unit dosage form in an appropriate vessel or container such as a glass vial, pre-filled syringe or other container that is hermetically sealed. In certain embodiments, the unit dosage form is provided as a sterile particulate free solution comprising a glycoprotein composition that is suitable for parenteral administration. In another embodiment, the unit dosage form is provided as a sterile lyophilized powder comprising a glycoprotein composition that is suitable for reconstitution.

[00304] In certain embodiments, the unit dosage form is suitable for intravenous, intramuscular, intranasal, oral, topical or subcutaneous delivery. Thus, the present invention encompasses sterile solutions comprising an arabinosylated glycoprotein suitable for each delivery route. The present invention further encompasses sterile lyophilized powders comprising an arabinosylated glycoprotein that are suitable for reconstitution.

[00305] As with any pharmaceutical product, the packaging material and container are designed to protect the stability of the product during storage and shipment. Further, the products include instructions for use or other informational material that advise the physician, technician or patient on how to appropriately prevent or treat the disease or disorder in question, as well as how and how frequently to administer the pharmaceutical. In other words, the article of manufacture includes instruction means indicating or suggesting a dosing regimen including, but not limited to, actual doses, monitoring procedures, and other monitoring information.

[00306] Specifically, the present invention provides an article of manufacture comprising packaging material, such as a box, bottle, tube, vial, container, pre-filled syringe, sprayer, insufflator, intravenous (i.v.) bag, envelope and the like; and at least one unit dosage form of a pharmaceutical agent contained within the packaging material, wherein the pharmaceutical agent comprises a liquid formulation containing a glycoprotein composition. The packaging material includes instruction means which indicate how that the glycoprotein composition can be used to prevent, treat and/or manage one or more symptoms associated with a disease or disorder.

[00307] The compounds, compositions, and methods described herein will be better understood by reference to the following examples, which are included as an illustration of and not a limitation upon the scope of the invention.

[00308] F. EXAMPLES

Sugars	D-arabinose D-altrose sucrose	D-arabinose	D-arabinose	D-arabinose
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[00309]

**Example 1:
Materials**

s & Methods

[00310] Cell Culture: Various Chinese Hamster Ovary (CHO) cell lines expressing various recombinant glycoproteins were evaluated in shaker flask cultures (see summary

Cell Line Name	Cell Line 1	Cell Line 2	Cell Line 3	Cell Line 4
Cell Line Type	CHO	CHO	CHO	CHO
Expressed Protein	mAb-1	DVD-1	mAb-2	mAb-3
Culture Vessel	Shaker Flasks	Shaker Flasks	Shaker Flasks	Shaker Flasks
Culture Mode	Fedbatch	Fedbatch	Fedbatch	Fedbatch
Initial Culture Temperature (°C)	36	36	36	36

above). All cultures utilized the same chemically-defined basal media, and chemically-defined feed media (CDFM), both of which contain D-glucose as the primary sugar. Each of the experimental conditions was supplemented with a variety of different sugars, including D-arabinose, D-altrose, or sucrose (Sigma Aldrich, St.Louis, MO) to evaluate for their potential impact on the resulting N-glycan oligosaccharide profile. In preparation of the cultures, the cell lines were expanded through separate seed train inoculums to generate enough cell biomass for inoculation of multiple cultures. Process conditions utilized during the cultures were similar between each experimental condition and the respective control condition (Table 1).

[00311] **Table 1:** Summary of novel sugar supplementation details

Sugar	0.1,1,10,20,50			
Concentrations	10	0.1,1,10,20,50	0.1,1,10,20,50	30
(mM) ^a	70			

[00312] Viable cell density (VCD) and cell viability values were measured through trypan blue exclusion via Cedex automated cell counters (Roche Applied Science, Indianapolis, IN), glucose and lactate values were measured with an ABL-805 (Radiometer Medical, Denmark) blood gas analyzer. Media pH measurements were also performed with an ABL-805 (Radiometer Medical, Denmark) blood gas analyzer. Media osmolality was measured on a Multi-Osmette 2430 osmometer (Precision Systems, Natick, MA).

[00313] Protein A Affinity Chromatography: Antibody titers were measured from clarified cell culture harvests on a Poros ATM (Life Technologies, Carlsbad, CA) affinity column using an HPLC system operating with a low pH, step elution gradient with detection at 280 nm. Absolute concentrations were assigned with respect to reference standard calibration curves. Purified antibodies subjected to additional analytical characterization were purified using MabSelectTM Protein A (GE Healthcare, Piscataway, NJ) using a low pH, step elution gradient, followed by buffer exchange using Spin-X® UF Concentrator columns (Corning Lifesciences, Tewksbury, MA), or equivalent, according to the manufacturer's recommended procedure.

[00314] N-glycan Oligosaccharide Profiling-LC/MS: The heavy chain of protein samples were analyzed using an HPLC (Agilent 1260, Santa Clara, CA) with a reversed phase column (Vydac, C4, 1 x 150 mm, 5μ particle size) coupled to a Q-TOF mass spectrometer (Agilent, 6510). Protein A-purified samples from cell culture harvests were first reduced using DTT at 37°C for 30 minutes. The samples were then loaded using 95% mobile phase A (0.08% formic acid and 0.02% TFA in water) and 5% mobile phase B (0.08% formic acid and 0.02% TFA in acetonitrile), and then eluted using a gradient of increasing levels of mobile phase B. The flow rate was 50 μL/min and the column temperature was set to 60°C. The mass spectrometer was operated in positive ion mode with an ion spray voltage of 4500 volts and a source temperature of 350°C. Resolved peaks centered on the known masses of different N-glycans were subsequently used to identify the different types of N-glycans, and the relative peak heights were used to quantitate their respective levels.

[00315] N-glycan Glycopeptide Mapping-LC/MS/MS: Protein samples were denatured with 6M guanidine-HCl at pH 8.0. The proteins were reduced with 10 mM DTT for 30 minutes at 37 °C. The proteins were alkylated with 25 mM iodoacetic acid for 30 minutes at 37 °C in the dark. The proteins were buffer exchanged to 10 mM tris pH 8.0 using NAP-5 columns. The proteins were digested with trypsin (1:20 trypsin:antibody) for 4 hours at 37 °C. The digest was quenched by adding 5 µL of formic acid. Peptide mapping was performed by LC/MS/MS using a Waters Acuity UPLC coupled to a Thermo Orbitrap Velos™. The summed spectrum was extracted for the chromatographic region where all the glycopeptides eluted. The relative peak intensities of all the glycovariants were determined as a percent of the total.

[00316] Rodent PK Study: Studies were conducted in accordance with the AbbVie IACUC guidelines. Sprague Dawley male rats (n=5 per group/dose route) were purchased from Charles River Laboratories (Kingston, NY) and were acclimated for at least 2 days before receiving a single subcutaneous injection or slow intravenous (IV) bolus dose injection of mAb-1. Blood from the lateral tail vein from each rat was collected via tail nick at 0.25, 4, and 24 hours, and then 2, 3, 7, 10, 14, 21, and 28 days post dose. All samples were stored at -80°C until further analysis. Serum concentrations of mAb-1 were measured using a GYROS method employing biotinylated antibodies to capture mAb-1, and Alexa Fluor 647 goat, anti-human IgG detection. Concentrations were calculated using logistic fit using XLfit4, and PK parameters calculated using non-compartmental analysis using PLASMA v2.6.12 (SParCS).

[00317] Dendritic Cell Uptake:

[00318] a. Isolation of monocytes, culture and stimulation: Peripheral blood mononuclear cells (PBMC) were isolated from leukopack of healthy donors by density gradient centrifugation over Ficoll-Paque (GE Healthcare, Pittsburgh, PA). Monocytes were isolated by magnetic sorting using CD14 microbeads (Miltenyi Biotec, San Diego, CA). The purity of the resulting monocytes, as assessed by flow cytometric analysis, was typically greater than 98%. Monocytes were cultured in RPMI1640 medium supplemented 2 mM L-glutamine, 100 ng/mL of recombinant human GM-CSF and 5 ng/mL of human IL-4 (Peprotech, Rocky Hill, NJ), 100 µg/mL penicillin, and streptomycin, and 10% fetal bovine serum at a density of 1×10^6 cells/mL at 37°C with 5% CO₂ for 5 days.

[00319] b. Dendritic cell differentiation and stimulation: Dendritic cells were generated by culturing monocytes in RPMI1640 medium supplemented with 100 ng/mL of

recombinant human GM-CSF and 5 ng/mL of human IL-4 (Peprotech, Rocky Hill, NJ) for 4 days. To investigate the TNF α production, dendritic cells were stimulated with 1 mg/mL LPS (from *Salmonella typhimurium*, Sigma-Aldrich, St. Louis, MO) for 1 hour.

[00320] c. Internalization assay: LPS stimulated dendritic cells were blocked with human IgG and stained with pH^{rodo} red labeled adalimumab (D2E7) on ice, then incubated at 37°C. As a negative control, an isotype matched control antibody was used. All the antibodies were conjugated with pH^{rodo} red using antibody labeling kit (Invitrogen) according to the manufacturer's protocol. Samples were analyzed on a Fortessa™ flow cytometer (Becton Dickinson, Franklin Lakes, NJ), and analysis was performed using Flowjo software (TreeStar Inc., Ashland, OR, USA).

[00321] Antigen Binding: Biacore® T100 (GE Healthcare Bio-Sciences, Pittsburgh, PA) was used to evaluate for differences in antigen binding between control and experimental samples. Approximately 5000 RU of polyclonal goat anti-human Fc IgG was immobilized on the flowcells of the biosensor chip. Purified samples from cell culture harvest were diluted as needed in HBS-EP buffer (GE Healthcare) to approximately the same concentrations. The samples were then captured on flow cells at a flowrate of 10 μ L/min for 60 seconds (s).. Dissociation was then monitored for 900s. Regeneration was performed after each run. Purified cell culture samples were compared to mAb-1 reference standard. All samples were assayed in triplicate.

[00322] Size Exclusion Chromatography: Protein A purified antibody samples from Cell Line 1 were diluted when necessary to 0.5-5 mg/mL in 1X PBS, and measured on a TSKgel® G3000SWXL column (Tosoh Bioscience, South San Francisco, CA) using an isocratic gradient on an HPLC with detection at 280 nm. High molecular weight (HMW), monomer, and low molecular weight (LMW) species were assigned and subsequently quantitated.

[00323] Viscosity Measurement: Taylor dispersion analysis (TDA) was performed with the Viscosizer 200 (Malvern) to determine sample viscosities. The specific viscosity for the sample solution was calculated using Poiseuille's Law and relied upon the accurate timing of UV absorbance migrating past two separate points within a capillary tube. Both the total length of the capillary and distance between the two observation points were known. By comparing the migration of a known sample against that of a reference buffer spiked with caffeine, the specific viscosity of protein formulations were determined. Ten μ L of samples were injected into a cellulose coated capillary under a continuous flow of

buffer (15mM histidine, pH 6.0) at 2,000 mBar of pressure. The same formulation buffer was utilized as the running buffer. Two detector heads were positioned in the capillary system and temperature was controlled at 25°C. The capillary was washed between experiments with buffer at 2,000 mBar of pressure as per the manufacturer's protocol. The UV detection wavelength utilized was 280 nm. The measured capillary ID / μm (Inner Diameter): 76.3 μm , length to end window 1, 45.0 cm, length to end window 2, 85.0 cm, total capillary length 130.0 cm.

[00324] Cytokine Release Assay: 96-well propylene plates were coated with 1 μg of purified protein from experimental samples in 60 μL of DPBS per well for 1.5 hrs at room temperature. After rinsing, 1×10^5 human peripheral blood mononuclear cells (PBMC) were added and incubated for approximately 2 days at 37°C. Well plate supernatants were then assayed for IL-1 β , IL-6, IL-8, IL-2, TNF α , and IFN γ using MSD (Meso Scale Discovery, Rockville, MD) technology. Values were measured using a SECTOR® Imager 6000 reader (Meso Scale Discovery, Rockville, MD). Experimental samples were compared to both positive and negative controls. A cytokine response was considered positive when the levels were greater than twice the level released from the negative control.

[00325] Complement Activation: 96-well EIA (Corning, Corning, NY) ELISA plates were coated with various concentrations of purified antibody protein from the experimental samples. Plate wells were then incubated with human serum from healthy donors as a source of complement for 1 hr at 37°C. Complement activation was determined by measuring the levels of human iC3 bound to the plate using mouse, anti-human iC3b antibody (Quidel, San Diego, CA), followed by incubation with anti-mouse IgG-HRP. Absorbance at 450 nm was read on a SpectraMax® 340PC plate reader (Molecular Devices, Sunnyvale, CA).

[00326] Fc γ R, FcR $_n$ Binding: Biacore® was used to evaluate for differences in binding of purified mAb-1 towards both human Fc γ Rs and human FcR $_n$ between control and experimental samples. For the Fc γ R binding assays, the following Fc γ Rs were immobilized on a CM5 chip using immobilized goat anti-histidine antibody: huFc γ RIIa138H-His, huFc γ RIIa131R-His, huFc γ RIIb-His, huFc γ RIIIa158F-His, huFc γ RIIIa158V-His. Experimental purified samples were then added to the chip over the following of cAntibody concentrations: 31.25-40,000 nM for Fc γ RII receptors and 7.8-2,000 nM for Fc γ RIIIa receptors, and the binding results monitored using a Biacore® T200 instrument (GE Healthcare). For the FcR $_n$ binding assays, the experimental purified

samples were directly immobilized to a CM5 chip. huFcRn was then added over a range of protein concentrations of 0.9 – 6,000 nM, and the binding results monitored by the Biacore® T200 instrument. All Biacore® kinetic experiments were performed with the use of the HBS-EP+ buffer system (GE Healthcare) at a flow rate of 50 µl/min. The association rate was monitored for 1-2 minutes followed by a 1-5 minute dissociation phase. Raw results were analyzed with the use of the Biacore® T200 Evaluation software version 2.0 (GE Healthcare).

[00327] Cell Lysis Assay for ADCC Measurement: ADCC activity was assessed using a calceinAM-based assay. Briefly, DoHH2 target cells were incubated for 30 minutes with 10-15 µM calceinAm in DMSO at a concentration of 4×10^6 cell/mL at 37°C, washed two times with culture medium, and then incubated with antibodies for 15 minutes on ice. The cells were then plated at 10,000/well in a 96-well v-bottom plate. NK-92 V158 or NK-92 F158 effector cells (E) were added at a 9:1 E:T ratio. After a 2.5-hour incubation at 37°C, 100 µL of the supernatant was collected from each well after spinning the plate at 1,300 RPM for five minutes, and released calcein was counted with an EnVision® Multilabel Reader (Perkin-Elmer). Triplicate wells were set up for each experimental condition. Background fluorescence due to medium or triton was subtracted from all data values. The results were then expressed as the percentage of lysis, calculated using the following formula:

$$\text{Specific Lysis (\%)} = \frac{(\text{experimental release} - \text{spontaneous release})}{(\text{maximum release} - \text{spontaneous release})} \times 100$$

where experimental release represents the mean relative fluorescence units (RFU) for the target cells in the presence of effector cells; spontaneous release represents the mean RFU for target cells incubated without effector cells; and maximal release represents the mean RFU for target cells incubated with 1% Triton X-100 (Fluka #93426).

[00328] CD16 (FcγRIIIa) Reporter Assay: CD16 signaling was assessed using CD16 transfected reporter cells (Promega, Madison, WI). Reporter cells and human, transmembrane-TNFα expressing HEK293 cells were incubated in a 96-well plate with increasing concentrations of antibody for 18 hours at a ratio of 1 reporter to 2 targets. After incubation, CD16-mediated luciferase induction was measured by adding Bio-Glo™ luciferase substrate (Promega, Madison, WI). Relative luciferase units were measured on an EnVision® 2103 Multilabel Reader (Perkin Elmer, Hopkinton, MA). Data were graphed using GraphPad Prism software (GraphPad Software, La Jolla, CA).

[00329] Statistics: Experimental results are expressed as the mean for those results generated from at least 3 independent values. Experimental results are expressed as is for those results generated from 1 value.

[00330] Abbreviations: Certain terms are referred to using abbreviations as described in Table 2.

[00331] **Table 2:** Abbreviations used in the Examples

ADCC	Antibody dependent cellular cytotoxicity
CHO	Chinese Hamster Ovary cells
Fuc	Fucose
FucT	Glycoprotein 6- α -L-fucosyltransferase
Gal	Galactose
GalT	β -N-acetylglucosaminyl glycopeptide β -1,4-galactosyltransferase
GlcNAc	N-acetylglucosamine
GnTI	α -1,3-mannosyl-glycoprotein 2- β -N-acetylglucosaminyltransferase
GnTII	α -1,6-mannosyl-glycoprotein 2- β -N-acetylglucosaminyltransferase
HMW	High molecular weight species
LC-MS	Liquid chromatography-mass spectrometry
LMW	Low molecular weight species
Man	Mannose
ManI	Mannosyl-oligosaccharide 1,2- α -mannosidase
ManII	Mannosyl-oligosaccharide 1,3-1,6- α -mannosidase
N/A	Not applicable
NK	Natural killer cells
PK	Pharmacokinetics
SEC	Size exclusion chromatography
VCD	Viable cell density

[00332] **Example 2: Cell Culture Performance (Cell Line 1)**

[00333] CHO cell line 1 expressing mAb-1 (adalimumab; see Table A) was evaluated in shaker flasks in fed-batch operation mode. Variable amounts of D-arabinose were supplemented into both the chemically-defined basal and feed medias. The cell culture performance results are highlighted in Figure 3. The viable cell density (VCD) profiles suggest that 0.1, 1, and 10 mM concentrations introduce no adverse impact to either peak VCD, or culture longevity. At 20 mM and 50 mM concentrations, the cells also grow appreciably well, but with peak VCD values that were reduced compared to the unsupplemented control. Regardless of these results, however, the cell viability results across the respective conditions were highly similar to each other, with all culture durations lasting either 13 or 14 days, when viability dropped below 70%. Harvest titers from the

cultures evaluated across the range of D-arabinose concentrations showed a nominally lower value compared to the unsupplemented control, which became more appreciable as the D-arabinose concentration approached 50 mM, where the titers dropped by approximately 40%. For this particular cell line, one may make the conclusion that D-arabinose is well-tolerated as a media supplement, however at appreciably high supplementations, there is the potential that cell culture process performance can decrease. It is thus expected that its use would be very friendly towards larger volumetric scale GMP cultures

[00334] Cell culture harvests from the aforementioned cultures were Protein A purified and the mAb-1 samples were analyzed for their N-glycan profiles via LC-MS of the reduced protein. A representative spectrograph for an arabinose supplemented cultures is shown in Figure 4 compared to the unsupplemented control. One can see from the figure that there was a noticeable shift in mass for all the fucosylated N-glycan species by approximately 14 Da in the culture supplemented with arabinose. This decrease in mass was consistent with the difference in molecular weight between that of L-fucose (the native N-glycan sugar) with D-arabinose (Figure 2). These results suggest (and confirmed in follow-up experiments as outlined below) that the N-glycans were afucosylated, and replaced with arabinosylated N-glycans. The average results from all of the arabinose supplemented cultures are shown in Table 3.

[00335] **Table 3:** Average N-glycan Profiles from Arabinose Supplemented Cultures Compared to the Unsupplemented Control

D-Arabinose Conc. (mM)	Man5 (%)	Man6 (%)	G0A-GlcNAc (%)	G0F-GlcNAc (%)	G0 (%)	G0A (%)	G0F (%)	G1A (%)	G1F (%)	Total Afucosylation (%)
0	2.1	1.0	0	8.6	0	0	83.3	0	3.6	3.1
0.1	1.9	1.0	0	8.5	0	0	82.6	0	3.4	2.9
1	2.7	1.0	0	7.1	0	0	70.1	0	3.1	3.7
10	0	1.0	10.8	0	3.5	79.6	0	3.2	0	98.2
20	0	1.5	13.4	0	3.5	76.3	0	3.4	0	98.1
50	0	3.6	11.7	0	2.7	73.7	0	6.4	0	98.1

[00336] The collective results indicate that D-arabinose supplementation was effective at reducing the levels of high mannose N-glycans, especially the 1 and 10 mM conditions, and most notably with the Man5 species. Arabinose was also able to increase the levels of G0 type N-glycans, as well as completely replace fucose with arabinose. The

D-arabinose titration experiment also highlights that somewhere between 1 and 10 mM is the effective concentration at which protein arabinosylation begins to be observed. At higher concentrations than this, the N-glycans were fully arabinosylated, with no appreciable presence of fucose on any of the N-glycans. These results highlight how mannose levels can be glycomodulated to extremely low levels. These results highlight the ease with which complete removal of fucose can be achieved through this approach. By simply incubating cells in culture with sufficient quantities of this particular sugar is enough for approximately 100% removal of fucose, and swapping in of arabinose. This is highly unexpected since most literature reports have shown that when cells in culture are exposed to a particular sugar, or compounds which facilitate an enhanced addition of that sugar, there is typically not a 100% incorporation of that sugar on the N-glycan such that all of the N-glycans display that sugar (Gramer et al., *Biotechnol. Bioeng.* 108:1591-1602 (2011)). Agents for increasing levels of galactosylated N-glycans, such as supplementation of media with trace metals (e.g., manganese, cobalt, iron, and the like) and/or with sugars other than arabinose (e.g., altrose, galactose, turanose, melezitose, and the like), as well as methods for supplementation thereof (e.g., amounts, concentrations, and the like), are well known in the art (see, for example, U.S. Pat. Publ. Nos. 2015/0050693, 2014/0314745, 2014/0271633, and 2012/0195885; the entire contents of each of which published application is incorporated herein by this reference).

[00337] To confirm that this removal of core fucose, and subsequent replacement with arabinose was specific, ¹³C labeled D-arabinose was purchased (Sigma, St. Louis, MO), supplemented into the cell culture media at a concentration of 30 mM, and evaluated. Figure 5 highlights the differences in reduced LC-MS of the purified mAb-1. It is readily apparent that there was a +1 Da shift in masses of all the N-glycans which typically contain fucose and are now arabinosylated. These results thus prove that the incorporation of arabinose (a 5 carbon sugar) for fucose (a 6 carbon sugar) is a highly effective process. Arabinose's ability to serve as a replacement for fucose is likely attributable to its structural similarity to fucose (Figure 2). The stereochemistry at the C-2 to C-4 position is exactly the same. Due to the close chemical similarity between these two sugars, it is apparent that the 5 carbon containing arabinose is able to be used as an alternative substrate for this addition reaction onto the N-glycan as that of the 6 carbon containing fucose. The capability of using arabinose in this regard is not immediately obvious, even more so when considering that the native fucose is unique and exists preferentially in the L-form before addition onto the N-

glycan. Arabinose is more commonly found in nature in its L-form. The arabinose that was utilized in the present work was the D-form.

[00338] There are other sugars in nature which resemble D-arabinose, one of which is D-altrose (Figure 2). To investigate whether D-altrose could also be used to replace L-fucose on native N-glycans, CHO cell line 1 was cultured in chemically-defined media supplemented with 10 mM of D-altrose. Upon purifying mAb-1 from the culture milieu, LC-MS-MS glycopeptide mapping was used to analyze the oligosaccharide diversity on the principal N-glycosylation site of the molecule (Figure 6). Upon inspection of the results, and comparison of the relative peak intensities between both arabinosylated mAb-1, and the predominantly fucosylated mAb-1, it was found that D-altrose was actually leading to arabinosylation of mAb-1. In this particular case, it is likely that the D-altrose was being broken down (perhaps due to the metabolism of the CHO cell line) into D-arabinose, which was then subsequently added onto the molecule. This experiment hints at a novel means with which to conduct native N-glycan sugar replacement. That is, one may target the use of novel precursor sugars, which are then processed by cells in culture towards a different, and more active sugar, which can then be used to elicit the desired native N-glycan sugar replacement effect.

[00339] A final cell culture experiment utilizing Cell Line 1 was also performed in shaker flask culture to generate sufficient amount of protein to enable extended analytical and functional characterization of the expressed protein (Figure 7). In this particular experiment, 15 mM D-arabinose was supplemented into both the basal and feed medias, in addition to 70 mM sucrose. Sucrose was evaluated as a control condition to enable large quantities of high mannose N-glycans, as has been shown previously in Hossler et al., *Biotechnol. Prog.* 30:1419-1431 (2014). The results indicate that there was no adverse impact to cell growth from this relatively high concentration of D-arabinose supplementation, which was in contrast to the sucrose supplemented culture, which did significantly decrease overall cell growth at this relatively high concentration of this disaccharide. Cell viability profiles, however, were not adversely impacted across the different experimental conditions. The arabinose supplemented culture modestly decreased harvest titer compared to the unsupplemented control, and the sucrose supplemented culture had a harvest titer much lower than the unsupplemented control. Upon culture harvest, mAb-1 was purified from each of these 3 cultures, and subjected to a large number of tests to evaluate for both structural and functional impacts.

[00340] Table 4 highlights that both D-arabinose and sucrose were very effective at eliciting changes in the protein oligosaccharide profile compared to the unsupplemented control. Arabinose had a similar impact as that which was reported above. Sucrose had a very significant impact on high mannose type N-glycans, and was evaluated as a control condition for high mannose to be tested alongside the low mannose condition elicited through the arabinose treatment..

[00341] **Table 4:** N-glycan Profiles from Arabinose & Sucrose Supplemented Cultures Compared to the Unsupplemented Control

Condition	Man 5	G0A-GlcNAc	G0F-GlcNAc	G0	Man 6	G0A	G0F	Man 7	G1A	G1F	Man 8	G2A	G2F
Control	3.7	0	10.2	0	0.6	0	80.6	0	0	4.7	0	0	0.2
70 mM Sucrose	22.3	0	16.3	0	10.0	0	40.2	5.6	0	3.3	2.0	0	0.4
15 mM D-Arabinose	2.5	5.2	0	4.0	0	82.7	0	0	5.2	0	0	0.1	0

[00342] **Example 3: Cell Culture Performance (Cell Line 2)**

[00343] CHO cell line 2 expressing DVD-1 (ABT-482) was evaluated in shaker flasks in fedbatch operation mode. DVD-1 (ABT-482) is a TNF/IL-17-binding DVD-Ig having the following sequence:

DVD-1 (ABT-482) (Light Chain)

DIQMTQSPSSLSASVGDRVTITCRASQDISQYLNWYQQKPGKAPKLLIYYTSRLQSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQGNTWPPTFGQGTKLEIKRGGSGGG
GSGDIQMTQSPSSLSASVGDRVTITCRASSGIISYIDWFQQKPGKAPKRLIYATFDLA
SGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCRQVGSYPETFGQGTKLEIKRTVAA
PSVFIFPPSDEQLKSGTASVCLLNFPYFPAKRVQWKVDNALQSGNSQESVTEQDS
KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

DVD-1 (ABT-482) (Heavy Chain)

EVQLVQSGAEVKKPGASVKVSCKASGYTFANYGHIWVRQAPGQGLEWMGWINTY
TGKPTYAQKFQGRVTMTTDTSTSTAYMELSSLRSEDTAVYYCARKLFTTMDVTDN
AMDYWGQGTITVTVSSGGGGSGGGGSEVQLVQSGAEVKKPGSSVKVSCKASGYTF
TDYIEHWVRQAPGQGLEWMGVNDPESGGTFYNQKFDGRVTLTADESTSTAYMEL
SSLRSEDTAVYYCTRYSKWDSFDGMDYWGQGTITVTVSSASTKGPSVFPLAPSSKS
TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVTVPS
SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE

MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

Variable amounts of D-arabinose were supplemented into both the chemically-defined basal and feed medias. The cell culture performance results are highlighted in Figure 8. The VCD profiles indicate that across the range of tested concentrations of 0.1 mM to 50 mM, the majority of the cultures supported peak VCD's either on par, on only nominally lower, with that of the unsupplemented control. The expressed DVD was harvested from each of the respective cultures when cell viability dropped below 70%. Only one of the D-arabinose supplemented cultures had a harvest date earlier than the unsupplemented control, with the higher supplemented conditions of 20 mM and 50 mM actually supporting a longer culture duration before the harvest criteria was triggered. Regardless of the harvest timing, the harvest titers from each of the respective cultures were approximately the same, with the longer duration cultures actually increasing the collective amount of DVD-1. The cumulative results with CHO cell line 2 indicates that D-arabinose does not adversely impact cell culture performance across a wide range of concentrations.

[00344] Cell culture harvests from the aforementioned cultures were Protein A purified and the DVD-1 samples were analyzed for their N-glycan profiles via LC-MS of the reduced protein. The average results from all of the arabinose supplemented cultures are shown in Table 5.

[00345] **Table 5:** N-glycan Profiles from Arabinose Supplemented Cultures Compared to the Unsupplemented Control

D-Arabinose Conc. (mM)	Man5 (%)	Man6 (%)	G0A-GlcNAc (%)	G0F-GlcNAc (%)	G0 (%)	G0A (%)	G0F (%)	G1A (%)	G1F (%)	Total Afucosylation (%)
0	2.1	0	0	6.6	0	0	85.0	0	4.4	2.1
0.1	2.1	0	0	6.5	0	0	85.3	0	4.2	2.1
1	2.4	0	0	5.9	0	0	85.1	0	4.8	2.4
10	2.1	0.8	2.8	3.6	3.8	34.5	48.3	1.2	2.6	45.2
20	2.5	2.1	3.5	3.1	4.2	38.8	40.9	1.6	2.4	52.7
50	5.3	5.0	3.8	4.0	4.4	33.5	37.8	2.1	3.3	54.1

[00346] The collective results indicate that D-arabinose supplementation was slightly less effective at reducing the levels of high mannose N-glycans compared to the results with mAb-1. However, arabinose was able to elicit a similar effect of increasing the levels of G0 type N-glycans, as well as completely replacing fucose with arabinose. The D-arabinose

titration experiment also highlights that somewhere between 1 and 10 mM is the effective concentration at which protein arabinosylation begins to be observed. At higher concentrations than this, the N-glycans were fully arabinosylated, with no appreciable presence of fucose on any of the N-glycans.

[00347] Example 4: Cell Culture Performance (Cell Line 3)

[00348] CHO cell line 3 expressing mAb-2 (anti-CD20 mAb) was evaluated in shaker flasks in fed-batch operation mode. mAb-2 has the following sequence:

mAb-2 (Light Chain)

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFFQKPGSSPKPWIYATSNLASGV
PVRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLELKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

mAb-2 (Heavy Chain)

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPG
NGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYF
NVWGAGTTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKA
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYT
QKSLSLSPGK

Variable amounts of D-arabinose were supplemented into both the chemically-defined basal and feed medias. The cell culture performance results are highlighted in Figure 9. Across the range of tested D-arabinose concentrations from 0.1 mM to 50 mM, the supplement was well-tolerated. Peak viable cell densities for each of the supplementation conditions were on par on average with that of the unsupplemented control. Only the 50 mM supplementation condition demonstrated average VCD results that dropped slightly below that of the control. Cell viability results were all reasonably the same across all of the evaluated conditions, with the unsupplemented control condition demonstrating a slight drop (on average) in cell viability towards the very end of the cultures. All cultures were harvested regardless on Day 14. As a result, the harvest titers from each of the D-arabinose supplemented cultures were on average slightly higher than the unsupplemented control condition. These results with cell line 3 further highlight the benign nature of using D-

arabinose as a cell culture media supplement with respect to both cell growth and productivity.

[00349] Cell culture harvests from the aforementioned cultures were Protein A purified and the mAb-2 samples were analyzed for their N-glycan profiles via LC-MS of the reduced protein. The average results from all of the arabinose supplemented cultures are shown in Table 6.

[00350] **Table 6:** N-glycan Profiles from Arabinose Supplemented Cultures Compared to the Unsupplemented Control

D-Arabinose Conc. (mM)	Man5 (%)	Man6 (%)	G0A-GlcNAc (%)	G0F-GlcNAc (%)	G0 (%)	G0A (%)	G0F (%)	G1A (%)	G1F (%)	Total Afucosylation (%)
0	1.8	0	0	6.6	0.2	0	85.0	0	4.2	2.0
0.1	1.6	0	0	6.2	0.2	0	86.7	0	3.6	1.8
1	0	0	2.8	3.6	1.2	34.2	53.7	0.6	3.2	38.8
10	0	0	7.5	0	2.8	84.8	0	2.9	0	98.0
20	0	0	7.8	0	2.9	84.0	0	3.3	0	98.0
50	0	0	10.4	0	2.8	81.0	0	3.6	0	98.7

[00351] The collective results indicate that D-arabinose supplementation was very effective at reducing the levels of high mannose N-glycans, especially the Man5 and Man6 species. Similar to mAb-1 and DVD-1, arabinose was also able to facilitate increasing levels of G0 type N-glycans, as well as completely replacing fucose with arabinose. The D-arabinose titration experiment suggests that between 0.1 mM and 1 mM is the effective concentration at which protein arabinosylation begins to be observed. At the tested concentrations higher than this, the N-glycans were fully arabinosylated, with no appreciable presence of fucose on any of the N-glycans.

[00352] Example 5: Cell Culture Performance (Cell Line 4)

[00353] CHO cell line 4 expressing mAb-3 (MAK-199-AM1-QL mAb; see Table A) was evaluated in shaker flasks in fed-batch operation mode. Variable amounts of D-arabinose were supplemented into both the chemically-defined basal and feed medias. The cell culture performance results are highlighted in Figure 10. In this particular experiment only one concentration of D-arabinose was evaluated in the basal and feed media. The 30 mM D-arabinose supplemented cultures grow comparably to the unsupplemented control, with cell viabilities trending similarly up till harvest on Day 13. Titers measured from the

harvests suggests a modest 15% reduction in the D-arabinose supplemented cultures. Collectively, the results with CHO cell line 4 agree with the results from CHO cell line 1. That is, D-arabinose is well tolerated as a culture media supplement; however at appreciably high concentrations there is the potential to reduce harvest titers, albeit in a nominal manner in the case of CHO cell line 4.

[00354] Cell culture harvests from the aforementioned cultures were Protein A purified and the mAb-3 samples were analyzed for their N-glycan profiles via LC-MS of the reduced protein. The average results from all of the arabinose supplemented cultures are shown in Table 7.

[00355] **Table 7:** N-glycan Profiles from Arabinose Supplemented Cultures Compared to the Unsupplemented Control

D-Arabinose Conc. (mM)	Man 5 (%)	Man 6 (%)	G0-GlcN Ac (%)	G0A-GlcN Ac (%)	G0F-GlcN Ac (%)	G0 (%)	G0 A (%)	G0 F (%)	G1 A (%)	G1 F (%)	G2 A (%)	G2 F (%)	Total Afucosylation (%)
0	1.5	2.3	5.5	0	3.2	3.8	0	75.6	0	5.6	0	1.5	13.1
30	0	3.1	7.6	2.2	1.5	11.3	50.2	16.5	3.3	2.0	2.3	0	80

[00356] The results indicate that D-arabinose supplementation was effective at reducing the levels of Man5 species to a greater degree, and the overall high mannose species to a lesser degree. Similar to mAb-1, DVD-1, mAb-2, and mAb-3, arabinose was able to facilitate increasing levels of G0 type N-glycans, as well as replace fucose with arabinose to a very significant relative abundance. The fact that 100% replacement was not observed with Cell Line 4 could be due to the fact that this cell line simply requires more arabinose in the culture media for this to be observed. Thirty mM might not have been sufficiently high enough for full arabinosylation, and a more significant drop in overall high mannose levels, to be observed.

[00357] Example 6. Structural and Functional Testing of Arabinosylated Glycoproteins

[00358] Size Exclusion Chromatography: Purified samples of mAb-1 from the 15 mM D-arabinose, 70 mM sucrose study were subjected to size exclusion chromatography (SEC) analysis to evaluate if the oligosaccharide impact mediated through either arabinose or sucrose supplementation impacted the presence of either high molecular weight (HMW), or low molecular weight (LMW) species. The results are shown in Table 8.

[00359] **Table 8:** SEC Profiles from Arabinose & Sucrose Supplemented Cultures

Cell Culture Condition	HMW (%)	Monomer (%)	LMW (%)
Control	1.7	98.2	0.2
70 mM Sucrose	1.7	98.0	0.3
15 mM D-Arabinose	1.6	98.2	0.2

[00360] These aforementioned results indicate that there is no adverse impact to either HMW or LMW species upon the cell culture supplementation of either sucrose or arabinose, and the resulting changes in the protein glycosylation profiles associated thereof.

[00361] Viscosity Measurements: Purified samples of mAb-1, DVD-1, and mAb-2 were subjected to viscosity measurements after concentrating all to 50 mg/mL in the same formulation buffer (15 mM histidine, pH 6.0). The results are shown in Table 9.

[00362] **Table 9:** Measured Viscosities From 3 Arabinosylated Proteins

Sample	Absolute Viscosity (cP)
mAb-1 Control	1.152
mAb-1 Arabinose	1.242
DVD-1 Control	1.479
DVD-1 Arabinose	1.479
mAb-2 Control	1.299
mAb-2 Arabinose	1.317

[00363] There was relatively no change in viscosity observed for mAb-2 or DVD-1 control samples in comparison to their arabinosylated counter-parts. There was a slight elevation in viscosity observed between the arabinosylated mAb-1 protein compared to its control. The cumulative results indicate there is no significant impact towards viscosity of arabinosylated protein formulations, and what measured changes may exist, they are essentially very minimal.

[00364] Dendritic Cell Uptake: Dendritic cells are potent and professional antigen presenting cells that have a very important role towards both the innate and adaptive immune responses. Uptake of antigens by dendritic cells is important for the activation of their contribution to immunogenic responses. A specific type of dendritic cell, called an inflammatory dendritic cell, has been shown to appear during inflammation (Segura and Amigorena, Trends Immunol. 34:440-445 (2013)). Thus, any antigen capable of enhancing

or reducing dendritic cell uptake *in vitro* may be potentially correlated to the activation or attenuation of their immune response towards the antigen *in vivo*.

[00365] Purified mAb-1 from the 15 mM D-arabinose/70 mM sucrose experiment was evaluated in a dendritic cell uptake assay (Figure 11). The results indicate that the highly mannosylated mAb-1 mediated by sucrose correlates with an elevated dendritic cell uptake. Thus, this material would be anticipated to elevate dendritic cell antigen display activity, including any associated immunogenic and inflammatory responses associated as a result of this. The arabinosylated mAb-1 mediated by arabinose had a much lower level of high mannose type N-glycans. As a result, its uptake by dendritic cells was significantly lower compared to the control. Thus, it is believed that this material reduce dendritic cell antigen display activity, including any associated attenuation of immunogenic and inflammatory responses. The results indicate that the utility of the present methods to control protein mannosylation levels provides for methods to tune the therapeutic profile, and by decreasing the respective levels, enables the manufacturing of a more efficacious therapeutic.

[00366] FcγR/FcR_n Binding: Therapeutic proteins have been documented to bind to antigens on cell surfaces and elicit their ability to kill target cells either through antibody dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), and direct apoptosis. Fcγ receptors serve a variety of *in vivo* immune functions and are important for therapeutic proteins to elicit their desired effect. In particular, the ability to bind to FcγRIIIa receptors is important for therapeutic proteins to elicit ADCC activity. FcγRIIIa is the binding receptor on natural killer (NK) effector cells that antibodies bind to through their Fc region. Upon antigen binding these same antibodies are then able to bind the receptors on NK cells to elicit ADCC activity. Studies have shown that both the target side and the effector side interactions of antibodies are important for ADCC activity (Iida et al., BMC Cancer 9:58 (2009)). Studies have also shown that the docking between an antibody and NK effector cells is mediated through the carbohydrate interactions associated with both (Ferrara et al., Proc. Natl. Acad. Sci. USA 108:12669-12674 (2011)). Thus, changing the protein glycosylation profile of recombinant protein therapeutics, especially fucose levels, is believed to impact the resulting ADCC activity according to reports (Shields et al., J. Biol. Chem. 277:26733 (2002); Shinkawa et al., J. Biol. Chem. 278:3466-3473 (2003)).

[00367] Purified mAb-1 from the 70 mM sucrose/15 mM arabinose experiment was evaluated via Biacore® for FcγR binding, and the results are shown in Table 10.

[00368] **Table 10:** FcγR Binding of mAb-1 from Arabinose and Sucrose Supplemented Cell Cultures

Sample	huFcγRIIb K _D (x10 ⁻⁶ M)	huFcγRIIa131H K _D (x10 ⁻⁶ M)	huFcγRIIa131R K _D (x10 ⁻⁶ M)	huFcγRIIIa158F K _D (x10 ⁻⁷ M)	huFcγRIIIa158V K _D (x10 ⁻⁷ M)
Control	5.9	2.4	5.6	8.8	1.3
70 mM Sucrose	10	3.9	6.0	1.4	0.6
15 mM D- Arabinose	6.3	2.5	5.6	6.2	0.6

[00369] The results indicate that mAb-1 purified from the sucrose supplemented culture demonstrated a nominal decrease in binding to FcγRIIb as well as FcγRIIa131H, with no significant impact on FcγRIIa131R. The binding to both FcγRIIIa158F and FcγRIIIa158V both increased nominally. From the material purified from the arabinose supplemented cell culture, there were negligible impacts towards FcγRIIb, FcγRIIa131H, and FcγRIIa131R binding. The binding to both FcγRIIIa158F and FcγRIIIa158V increased as well. The fact that both the sucrose and arabinose supplemented cultures provided for glycoproteins that increased the association between mAb-1 and FcγRIIIa makes sense since both cultures provided for material that was considerably lower in fucose content. By elevating FcγRIIIa binding, these results are consistent with what is observed for an enhanced ADCC activity (Shields et al., J. Biol. Chem. 277:26733 (2002)). The defucosylation and arabinosylation of N-glycans elicited through the culture media supplementation of arabinose is thus demonstrated to provide for beneficial improvements for those therapeutic proteins with which ADCC activity is of principal interest.

[00370] In humans the FcRn receptor has been shown to be important for facilitating transport of IgG from mother to fetus, and it has also been implicated in IgG turnover (Roopenian and Akilesh, Nat. Rev. Immunol. 7:715-725 (2007)). Purified mAb-1 from the 15 mM arabinose/70 mM sucrose experiment was evaluated via Biacore® for FcRn binding, and the results are shown in Table 11.

[00371] **Table 11:** FcRn Binding of Antibody from Arabinose and Sucrose Supplemented Cell Cultures

Sample	huFcRn K _D (x10 ⁻⁶ M)
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Control	7.1
70 mM Sucrose	7.5
15 mM D-Arabinose	7.3

[00372] The results indicate that the antibodies purified from the sucrose and arabinose supplemented cell cultures did not have any net increase or decrease on FcRn binding. From the perspective of FcR_n binding, it thus stands to reason that these glycomodulated antibodies can be considered similar to each other.

[00373] Antigen Binding: mAb-1 is a neutralizing antibody that binds to its target antigen with strong affinity. Ordinarily mAb-1 is fucosylated, and in the 15 mM arabinose/70 mM sucrose experiment, it was found that the oligosaccharides were significantly impacted as highlighted above. To further characterize the functional impact of these oligosaccharide changes towards the antibody, antigen binding assays were performed via Biacore®. The results from this study are shown in Table 12.

[00374] **Table 12:** Antigen Binding of mAb-1 from Arabinose and Sucrose Supplemented Cell Cultures

Sample	Antigen Binding K _D (x10 ⁻¹¹ M)
Control	8.14
70 mM Sucrose	8.15
15 mM D-Arabinose	7.96

[00375] The results indicate that there was no adverse impact on antigen binding due to the various oligosaccharide changes incurred through cell culture supplementation of sucrose or arabinose. It thus stands to reason that these glycomodulated antibodies can be considered similar to each other with respect to antigen binding.

[00376] Cytokine Release: Cytokines are a broad class of proteins produced by immune cells, as well as non-immune cells such as fibroblasts. These proteins serve a great deal of functions, including humoral and cell-based immune response, and frequently modulate other target cells, or the cells that secrete the cytokines. Many cytokines are categorized as pro-inflammatory cytokines for their role associated with inflammation. Thus, monitoring the levels of these particular cytokines upon exposure to a specific compound provides an indication of the resulting levels of inflammatory responses associated with this exposure. In the present example, a series of inflammatory cytokines were monitored upon administration of various glycomodulated mAb-1 samples. The

results using PBMC cells from 3 different donors are shown below in Table 13. Positive results were assigned when the measured cytokine levels exceeded two times (2X) the levels elicited with the negative control (Herceptin).

[00377] Table 13: Cytokine Release Elicited with Antibody from Arabinose and Sucrose Supplemented Cell Cultures

	PBMC Source: Donor 1					
	Cytokines Measured (pg/mL)					
Cell Culture Media	IL-1 β	IFN γ	IL-2	IL-6	IL-8	TNF α
Experimental Control	8	2	16	41	12056	36
70 mM Sucrose	10	4	14	47	23594	60
15 mM D-Arabinose	15	3	N/A	40	19198	65
Negative Control	12	6	12	48	23172	223
	PBMC Source: Donor 2					
	Cytokines Measured (pg/mL)					
Sample	IL-1 β	IFN γ	IL-2	IL-6	IL-8	TNF α
Experimental Control	19	3	19	37	15886	68
70 mM Sucrose	29	4	17	44	20315	96
15 mM D-Arabinose	23	4	15	27	16726	104
Negative Control	24	9	69	38	22405	232
	PBMC Source: Donor 3					
	Cytokines Measured (pg/mL)					
Sample	IL-1 β	IFN γ	IL-2	IL-6	IL-8	TNF α
Experimental Control	10	12	N/A	25	6726	57
70 mM Sucrose	15	27	23	70	14554	89
15 mM D-Arabinose	15	17	N/A	37	6949	93
Negative Control	15	26	13	39	20334	305

[00378] These aforementioned results indicate that across the measured cytokines there were no significant changes compared to the unsupplemented control condition, that were also greater than two times the amount from the negative control. Hence, there was no adverse impact to the elicitation of inflammatory cytokines upon exposure to the protein material generated from either the sucrose or arabinose cell culture media supplementation.

[00379] Complement Activation: The complement system is an important part of the innate immune system that is comprised of a series of proteins which get activated by a variety of triggers, including the classical, alternative, and lectin complement pathways. Upon activation, proteases in these pathways degrade pathway-specific proteins which

leads to further molecular events which cascade and amplify leading to cell lysis or opsonization of foreign cells, as well as other immune defensive responses.

[00380] In the present work, it was investigated whether glycomodulated mAb-1 elicited through 15 mM arabinose and 70 mM sucrose cell culture media supplementation facilitated any change towards human complement activation. The results are shown in Figure 12 using serum from two different donors. Figure 12 demonstrates that complement activation elicited from mAb-1 generated through either sucrose or arabinose cell culture supplementation was not any higher than complement activation elicited from an antibody generated through the unsupplemented experimental control. Indeed, the results shown in Figure 12 demonstrate that arabinosylation preserves the ability of a therapeutic antibody to activate complement in contrast to the well-known problem that removal of fucosyl residues compromises the ability of a therapeutic antibody to activate complement (Dalle et al., Mol. Cancer Therap. 10:178-185 (2011); Illidge et al., Cancer Treat. Rev. 41:784-792 (2015)). Thus, it can be concluded that there was no adverse impact to complement activation as a result of either of these glycomodulation techniques.

[00381] Rodent Pharmacokinetics (PK): Rat PK studies were conducted to evaluate if there was any impact towards the *in vivo* clearance rate of either of the arabinosylated antibodies purified from the 15 mM arabinose supplemented cell culture samples. Clearance rates were compared to the antibody purified from the unsupplemented experimental control and the results are shown in Figure 13 and Table 14.

[00382] **Table 14:** Rat PK Results with Antibody from Arabinose Supplemented Cell Culture

Sample	Intravenous			Subcutaneous		
	T _{1/2} (day)	CL (mL/h/kg)	V _{ss} (mL/kg)	T _{1/2} (day)	C _{max} (µg/mL)	F (%)
Control	12.3 +/- 2.0	0.23 +/- 0.01	88.8 +/- 11.6	11.9 +/- 1.4	41.1 +/- 8.7	78.5 +/- 19.1
15 mM D-Arabinose	16.2 +/- 4.3	0.11 +/- 0.02	55.1 +/- 10.6	11.5 +/- 2.0	58.9 +/- 7.9	58.3 +/- 5.4

[00383] These aforementioned cross-species results (human antibody in a rat model), as well as the standard deviations centered on the mean results, indicate that there was on average a nominally longer half-life with the arabinosylated sample compared to the unsupplemented control in the IV administered material, with the corresponding means +/- 1 SD overlapping each other. In the case of the subcutaneously administered material, the

mean half-lives and corresponding overlapping +/- 1 SD values were comparable. Hence, there was no adverse impact towards circulatory clearance introduced as a result of the arabinosylation of the antibody. This is an unexpected conclusion, since it has been commonly observed that the introduction of non-natively displayed N-glycan sugars can induce an immune response in non-native species, such as what has been shown with $\alpha(1,3)$ linked galactose moiety exposure in humans (Jenkins and Curling, *Enz. Microb. Technol.* 16:354-364 (1994); Jenkins et al., *Nat. Biotechnol.* 14:975-981 (1996)).

[00384] Antibody Dependent Cellular Cytotoxicity (ADCC) and Fc γ RIIIa Signaling: The cumulative effect on N-glycosylation through media supplementation of D-arabinose is very significant due to its role in modulating those particular sugars which have been shown in the literature to be important for immunogenicity, PK, and ADCC. Decreasing the levels of mannose is potentially beneficial due to mannose's potential role towards facilitating higher immunogenicity as well as shorter circulatory PK as described above. Removing fucose is beneficial, because there are many reports in the literature that have demonstrated how fucose removal is positively correlated to an increase in ADCC activity. Collectively, the impact of arabinose on protein glycosylation profiles suggests that there are numerous impacts on attributes that are associated with literature validated improvements in therapeutic efficacy. mAb-2 is an oncology relevant recombinant protein that has ADCC as a principal component of its method of action. Since it was proven that mAb-2 was very responsive towards a high degree of arabinosylation, and this facilitated an equal amount of overall afucosylation, mAb-2 was purified and analyzed with a cell based ADCC assay following the procedure as outlined in Example 1 (Figure 14). The results indicate a very similar behavior between both the higher affinity and lower affinity allele of Fc γ RIIIa evaluated as part of the assay. That is, with the exception of the lowest arabinose supplemented culture, the mAb-2 generated from the cultures supplemented with more than 0.1 mM arabinose increased ADCC activity in a very significant manner. The higher arabinosylated material facilitated the largest reductions in fucose, which also facilitated the largest increases in ADCC activity. These results are consistent with what one would expect through the enhancement of Fc γ RIIIa binding, as which was demonstrated with arabinosylated mAb-1. In previous reports, the removal of fucose has been correlated towards the increase in ADCC activity. However, in the present case, mAb-2 wasn't just fucose-deficient, the sugar was replaced with another sugar, and a smaller sugar at that. It is believed through this study that the complete removal of fucose is not completely necessary

to elicit this increase in ADCC activity. Simply removing one methyl group from fucose (i.e., arabinose) is sufficient to elicit higher ADCC, which is unexpected from the state of the art. Moreover, mAb-1 also unexpectedly preserved the ability of the therapeutic antibody to activate complement in contrast to the well-known problem that removal of fucosyl residues compromises the ability of a therapeutic antibody to activate complement.

[00385] mAb-3 binds TNF α and is an immunology relevant recombinant protein. The antibody was proven to be very responsive towards protein arabinosylation, and was thus purified and analyzed for CD16 (Fc γ RIIIa) signaling (Figure 15). An elevated signal from the assay would indicate a higher binding towards CD16, and would reinforce the previous results that glycoproteins whose mechanism of action relies on CD16 binding (e.g., ADCC), could be enhanced through arabinosylation. Arabinosylated mAb-3 and predominantly fucosylated mAb-3 (control) were purified and compared alongside reference material from mAb1 that was both predominantly fucosylated, as well as afucosylated through the addition of kifunensine, a small molecule which inhibits the ManI enzyme and facilitates high mannose N-glycans. The results from both the high and low affinity allele of CD16 suggest that the arabinosylated mAb-3 significantly enhanced the binding towards CD16. The results also suggest that signaling through CD16 from the arabinosylated mAb3 approximates that of the afucosylated mAb1. It is believed that protein arabinosylation can support an enhancement in signaling through CD16 that is competitive towards the response seen with the complete removal of fucose.

[00386] In the foregoing examples, it has been found that supplementation of the sugars arabinose and sucrose into the culture media of mammalian cells is effective towards the re-distribution of protein glycosylation profiles. In the case of sucrose, this has been documented previously (Hossler et al., Biotechnol. Prog. 30:1419-1431 (2014)). Sucrose has a proven role towards the targeted increase in high mannose type N-glycans and is shown as a negative control in the foregoing examples for the glycomodulation events incurred through the use of arabinose. The use of the arabinose monosaccharide as a cell culture media supplement for the targeted shifting of protein glycosylation profiles was demonstrated to provide for numerous changes towards the protein glycosylation profile of proteins, including recombinant proteins. Arabinose was shown to lead to a significant reduction in high mannose type N-glycans, an increase in G0 type N-glycans, and either the complete or predominant removal of fucose sugars on N-glycans with arabinose being inserted in its place. Through the use of multiple recombinant cell lines it was demonstrated

that purified recombinant proteins utilizing these glycomodulation tools provides for therapeutic benefits as described above. mAb-1 purified from arabinose supplemented cultures demonstrated a significant decrease in dendritic cell uptake, an increase in FcγRIIIa binding consistent with the mechanism for an increased ADCC response, as well as a comparable circulatory PK in a rat model. The demonstrated utility of this approach towards recombinant protein production, as well as therapeutic benefit associated thereof, enable the manufacturing of more efficacious and effective protein based biologics.

[00387] The capability of utilizing D-arabinose as a replacement sugar for L-fucose is an important observation and tool for enabling glycomodulation. It also highlights exactly which carbon positions on a sugar are principally important for the ability of the α(1,6) FucT enzyme, as well as the associated GDP-arabinose biosynthetic enzymes, to recognize arabinose as a substrate. In this regard, it is apparent that carbon positions 1, 2, 3, and 4 are principally important for these corresponding enzymatic reactions to proceed. It is believed that other sugars which preserve the stereochemistry at these positions will behave similarly to the D-arabinose example described herein. Fucose is unique amongst the sugars that comprise a typical N-glycan in the sense that it is an L-sugar, whereas the others are D-sugars. In the case of arabinose, it was demonstrated that D-arabinose was effective at eliciting changes in the protein glycosylation profile, including the replacement of L-fucose. Since arabinose is more typically found in nature in its L-form, the utility of the D-arabinose sugar in the present methods is unexpected.

[00388] The use of D-arabinose as a novel substrate for the FucT enzyme for addition onto N-glycans is also unexpected given the state of the art since protein glycosylation enzymes in mammalian cells are generally accepted to exhibit strong specificity for both the glycosyl donor and the protein acceptor substrates (Breton et al., *Glycobiol.* 16:29R-37R (2006)). The results described herein show that this is not necessarily always true when it comes to the enzymatic activity of the FucT enzyme, which is capable of recognizing its native 6-carbon fucose substrate, in addition to the smaller 5-carbon D-arabinose substrate.

[00389] Example 5. Arabinosylated Glycoprotein Production

[00390] By the selective supplementation of manganese and arabinose into either the basal or feed media of mammalian cells in culture, a targeted oligosaccharide optimization of glycoproteins, such as adalimumab, is demonstrated.

[00391] The cell line used to express this aforementioned recombinant glycoprotein was a clonally derived Chinese Hamster Ovary (CHO) cell line of DUKX-B11 origin, transfected with a proprietary plasmid vector encoding adalimumab (mAb-1) using standard promoters and selection systems such as dihydrofolate reductase (dhfr). Cryopreserved cells from a research cell bank of Cell Line 1 are thawed and expanded into increasingly larger volumes using sterile, plastic shaker flasks. Cell 1 was evaluated in shaker flasks in fed-batch operation mode. Variable amounts of D-arabinose were supplemented into both the chemically-defined basal and feed medias. Cell growth and antibody productivity were compared between these cultures with that of the unsupplemented control (Figure 16). The results indicate that between the evaluated concentrations of 0.1 mM to 10 mM there was no adverse impact towards cell growth, or cell viability. Cell viability results between the D-arabinose supplemented cultures and the control indicate that cell viability was also not adversely impacted. Antibody titers at harvest were similar across all the respective cultures. These aforementioned results indicate that D-arabinose is well tolerated by CHO cells in culture with respect to both cell growth and recombinant protein productivity. It is thus expected that its use would be very friendly towards larger volumetric scale GMP cultures due to the lack of any adverse impact. For comparison purposes, an additional culture was performed combining both 10 mM D-arabinose and 1 μ M MnCl₂ supplementation into both the basal and feed medias. The purpose of this culture was to compare the impact to the oligosaccharide profiles from just D-arabinose alone with that of additional Mn, a known modulator for increasing G1/G2 type N-glycans, to ascertain for any combinatorial type impact. The full results from all of the variably supplemented cultures are shown in Table 15.

Table 15: N-glycan Profiles from Arabinose Supplemented Cultures Compared to the Unsupplemented Control

D-Arabinose Conc. (mM)	Mn Conc. (μ M)	Man5	G0A-GlcNAc	G0F-GlcNAc	G0	Man6	G0A	G0F	G1A	G1F	G2A	G2F
0	0	6.4	0	10.8	0	0	0	75.5	0	4.9	0	0.5
0.1	0	6.2	0	9.9	0	2.0	0	74.1	0	4.7	0	0.6
1	0	5.5	5.5	6.7	2.1	1.4	24.1	47.3	1.5	3.4	0.5	0
10	0	3.5	9.0	0	4.4	0	75.6	0	5.1	0.8	0	0
10	1	2.0	4.9	0.3	6.5	0	55.5	0	26.1	0	3.8	0

The collective results indicate that D-arabinose supplementation was very effective at reducing the levels of high mannose N-glycans, increasing the levels of G0 and G1/G2 type N-glycans, as well as completely replacing fucose with arabinose. The D-arabinose titration experiment also highlights that somewhere between 0.1 and 1.0 mM is the effective concentration at which arabinosylation begins to be observed. At higher concentrations than this, the N-glycans were fully arabinosylated, with no appreciable presence of fucose on any of the N-glycans. The media supplementation of manganese in addition to the 10 mM D-arabinose facilitated the largest overall increase of galactosylated N-glycans. However, this condition also facilitated the largest overall decrease in high mannose type N-glycans, suggesting that the D-arabinose and Mn had a combinatorial effect towards this reduction. These results are important in the sense that they highlight how mannose levels can be glycomodulated to extremely low levels, and that the use of D-arabinose as a cell culture media supplement is also compatible with other glycomodulation methods. These results are also important because they highlight the ease with which complete removal of fucose can be achieved through this approach.

[00392] Both the production bioreactor growth media and feed media are supplemented using manganese and D-arabinose to elicit the desired impact, and optimize the resulting oligosaccharide profile in the expressed protein. The means with which this oligosaccharide optimization can be accomplished included the supplementation of manganese levels in the range of 0 to 100 μ M, the supplementation of galactose levels in the range of 0 to 50 mM, and the supplementation of arabinose in the 0 to 50 mM range. Through the selective media supplementation of each of these media components, it is demonstrated that the cumulative high mannose type N-glycans (i.e., Man5, Man6, Man7, Man8, and Man9) can be reduced to nominally low levels (e.g., < 2%), the levels of galactosylated N-glycans (G1A, G2A) can be specifically increased to levels >30%, and the levels of afucosylation can be increased to levels > 99%.

[00393] The foregoing description of the present invention provides illustration and description, but is not intended to be exhaustive or to limit the invention to the precise one disclosed. Modifications and variations are possible in light of the above teachings or may be acquired from practice of the invention. Thus, it is noted that the scope of the invention is defined by the claims and their equivalents.

What is claimed is:

1. A recombinant glycoprotein comprising at least one N-linked glycan, wherein the N-linked glycan comprises at least one arabinose residue.
2. The recombinant glycoprotein of claim 1, wherein the N-linked glycan is selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc.
3. The recombinant glycoprotein of claim 1 or 2, wherein the at least one arabinose residue is attached to a core GlcNAc residue of the N-linked glycan.
4. The recombinant glycoprotein of any one of claims 1-3, wherein the recombinant glycoprotein does not comprise a fucose residue attached to a core GlcNAc residue of the N-linked glycan.
5. The recombinant glycoprotein of claim 4, wherein the recombinant glycoprotein does not comprise a fucose residue.
6. The recombinant glycoprotein of any one of claims 1-5, wherein the glycoprotein is an antibody or antigen-binding fragment thereof.
7. The recombinant glycoprotein of claim 6, wherein the antibody or antigen-binding fragment thereof is a monoclonal antibody or antigen-binding fragment thereof, optionally wherein the monoclonal antibody or antigen-binding fragment thereof comprises a light chain variable region sequence and a heavy chain variable region sequence selected from the group consisting of light chain variable region and heavy chain variable region sequences shown in Tables A and B.
8. The recombinant glycoprotein of any one of claims 1-7, wherein one or more pharmacophores is conjugated to at least one of the at least one arabinose residues.
9. The recombinant glycoprotein of claim 8, wherein at least one of the one or more pharmacophores is conjugated to at least one of the at least one arabinose residues using a chemical linker.

10. A composition comprising a population of one or more glycoforms of a recombinant glycoprotein, wherein at least one of the one or more glycoforms in the composition comprises at least one N-linked glycan, wherein the N-linked glycan comprises at least one arabinose residue.

11. The composition of claim 10, wherein the N-linked glycan is selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc.

12. The composition of claim 10 or 11, wherein the arabinose residue is attached to a core GlcNAc residue of the N-linked glycan.

13. The composition of any one of claims 10-12, wherein at least one of the one or more glycoforms in the composition does not comprise a fucose residue.

14. The composition of claim 13, wherein the at least one of the one or more glycoforms in the composition does not comprise a fucose residue attached to a core GlcNAc residue of the N-linked glycan.

15. The composition of any one of claims 10-14, wherein at least one of the one or more glycoforms in the composition comprises an arabinose residue and does not comprise a fucose residue.

16. The composition of claim 15, wherein the at least one of the one or more glycoforms in the composition comprises at least one arabinose residue attached to a core GlcNAc residue of the N-linked glycan and does not comprise a fucose residue at the position of an arabinose attached to the core GlcNAc residue of the N-linked glycan.

17. The composition of any one of claims 10-16, wherein the glycoprotein is an antibody or antigen-binding fragment thereof.

18. The composition of claim 17, wherein the antibody or antigen-binding fragment thereof is a monoclonal antibody or antigen-binding fragment thereof, optionally wherein the monoclonal antibody or antigen-binding fragment thereof comprises a light chain variable region sequence and a heavy chain variable region sequence selected from the group consisting

of light chain variable region and heavy chain variable region sequences shown in Tables A and B.

19. The composition of any one of claims 10-18, wherein at least 1% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue.

20. The composition of claim 19, wherein at least 1% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue attached to an N-linked glycan selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc.

21. The composition of any one of claims 10-20, wherein at least 1% of the glycoprotein in the composition comprises at least one arabinose residue attached to a core GlcNAc residue of the N-linked glycan and does not comprise a fucose residue at the position of an arabinose attached to the core GlcNAc residue of the N-linked glycan.

22. The composition of any one of claims 10-21, wherein less than 54% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue.

23. The composition of any one of claims 10-22, wherein less than 54% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue attached to a core GlcNAc residue of the N-linked glycan.

24. The composition of any one of claims 10-23, wherein less than 20% of the glycoprotein in the composition comprises a glycoform comprising five or more mannose residues.

25. The composition of any one of claims 10-24 produced by culturing a host cell expressing the recombinant glycoprotein in cell culture media supplemented with D-arabinose and/or D-altrose, optionally wherein the host cell is a CHO cell.

26. The composition of claim 25, wherein D-arabinose and/or D-altrose is supplemented at about 0.1 mM or greater of the cell culture media.

27. The composition of claim 26, wherein D-arabinose and/or D-altrose is supplemented between about 0.1 mM and about 100 mM of the cell culture media.

28. The composition of claim 27, wherein D-arabinose and/or D-altrose is supplemented at about 15 mM of the cell culture media.

29. The composition of any one of claims 10-28 produced by culturing a host cell expressing the recombinant glycoprotein in cell culture media supplemented with D-arabinose and/or D-altrose and at least one additional agent to 1) increase the proportion of glycoforms comprising an N-linked glycan selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc and 2) decrease the proportion of glycoforms comprising five or more mannose residues.

30. The method of claim 29, wherein the at least one additional agent is a trace metal, or a sugar other than arabinose.

31. The method of claim 30, wherein the trace metal is selected from the group consisting of manganese, cobalt, and iron.

32. The method of claim 30, wherein the sugar other than arabinose is selected from the group consisting of altrose, galactose, turanose, and melezitose.

33. The composition of any one of claims 25-32, wherein the composition has at least one or more selected from the group consisting of an increased half-life, decreased immunogenicity, maintained or increased complement activation, maintained or increased complement dependent cytotoxicity (CDC) activity, and increased antibody-dependent cellular cytotoxicity (ADCC) activity as compared to a composition produced by culturing the host cell expressing the recombinant glycoprotein in cell culture media not supplemented with the D-arabinose and/or D-altrose.

34. The composition of any one of claims 10-33, wherein one or more pharmacophores is conjugated to at least one of the at least one arabinose residues.

35. The composition of claim 34, wherein at least one of the one or more pharmacophores is conjugated to at least one of the at least one arabinose residues using a chemical linker.

36. A method of producing a composition comprising a population of one or more glycoforms of a recombinant glycoprotein, wherein at least one of the one or more glycoforms in the composition comprises at least one N-linked glycan, wherein the N-linked glycan comprises at least one arabinose residue, comprising culturing a cell expressing the recombinant glycoprotein in cell culture media supplemented with D-arabinose and/or D-altrose, optionally wherein the host cell is a CHO cell.

37. The method of claim 36, wherein the N-linked glycan is selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc.

38. The method of claim 36 or 37, wherein the arabinose residue is attached to a core GlcNAc residue of the N-linked glycan.

39. The method of any one of claims 36-38, wherein at least one of the one or more glycoforms in the composition does not comprise a fucose residue.

40. The method of claim 39, wherein the at least one of the one or more glycoforms in the composition does not comprise a fucose residue attached to a core GlcNAc residue of the N-linked glycan.

41. The method of any one of claims 36-40, wherein at least one of the one or more glycoforms in the composition comprises an arabinose residue and does not comprise a fucose residue.

42. The method of claim 41, wherein the at least one of the one or more glycoforms in the composition comprises at least one arabinose residue attached to a core GlcNAc residue of the N-linked glycan and does not comprise a fucose residue at the position of an arabinose attached to the core GlcNAc residue of the N-linked glycan.

43. The method of any one of claims 36-42, wherein the glycoprotein is an antibody or antigen-binding fragment thereof.

44. The method of claim 43, wherein the antibody or antigen-binding fragment thereof is a monoclonal antibody or antigen-binding fragment thereof, optionally wherein the monoclonal antibody or antigen-binding fragment thereof comprises a light chain variable region sequence and a heavy chain variable region sequence selected from the group consisting of light chain variable region and heavy chain variable region sequences shown in Tables A and B.

45. The method of any one of claims 36-44, wherein at least 1% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue.

46. The method of claim 45, wherein at least 1% of the glycoprotein in the composition comprises a glycoform comprising at least one arabinose residue attached to an N-linked glycan selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc.

47. The method of any one of claims 36-46, wherein at least 1% of the glycoprotein in the composition comprises at least one arabinose residue attached to a core GlcNAc residue of the N-linked glycan and does not comprise a fucose residue at the position of an arabinose attached to the core GlcNAc residue of the N-linked glycan.

48. The method of any one of claims 36-47, wherein less than 54% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue.

49. The method of any one of claims 36-48, wherein less than 54% of the glycoprotein in the composition comprises a glycoform comprising a fucose residue attached to a core GlcNAc residue of the N-linked glycan.

50. The method of any one of claims 36-49, wherein less than 20% of the glycoprotein in the composition comprises a glycoform comprising five or more mannose residues.

51. The method of any one of claims 36-50, wherein D-arabinose and/or D-altrose is supplemented at about 0.1 mM or greater of the cell culture media.

52. The method of claim 51, wherein D-arabinose and/or D-altrose is supplemented between about 0.1 mM and about 100 mM of the cell culture media.

53. The method of claim 52, wherein D-arabinose and/or D-altrose is supplemented at about 15 mM of the cell culture media.

54. The method of any one of claims 36-53 further comprising culturing the host cell expressing the recombinant glycoprotein in cell culture media supplemented with at least one additional agent that 1) increases the proportion of glycoforms comprising an N-linked glycan selected from the group consisting of G0A, G1A, G2A, G0A-GlcNAc, and G1A-GlcNAc and 2) decreases the proportion of glycoforms comprising five or more mannose residues.

55. The method of claim 54, wherein the at least one additional agent is a trace metal, or a sugar other than arabinose.

56. The method of claim 55, wherein the trace metal is selected from the group consisting of manganese, cobalt, and iron.

57. The method of claim 56, wherein the sugar other than arabinose is selected from the group consisting of altrose, galactose, turanose, and melezitose.

58. The method of any one of claims 36-57, wherein the composition has at least one or more selected from the group consisting of an increased half-life, decreased immunogenicity, maintained or increased complement activation, maintained or increased complement dependent cytotoxicity (CDC) activity, and increased antibody-dependent cellular cytotoxicity (ADCC) activity as compared to a composition produced by culturing the host cell expressing the recombinant glycoprotein in cell culture media not supplemented with the D-arabinose and/or D-altrose.

59. The method of any one of claims 36-58, wherein one or more pharmacophores is conjugated to at least one of the at least one arabinose residues.

60. The method of claim 59, wherein at least one of the one or more pharmacophores is conjugated to at least one of the at least one arabinose residues using a chemical linker.

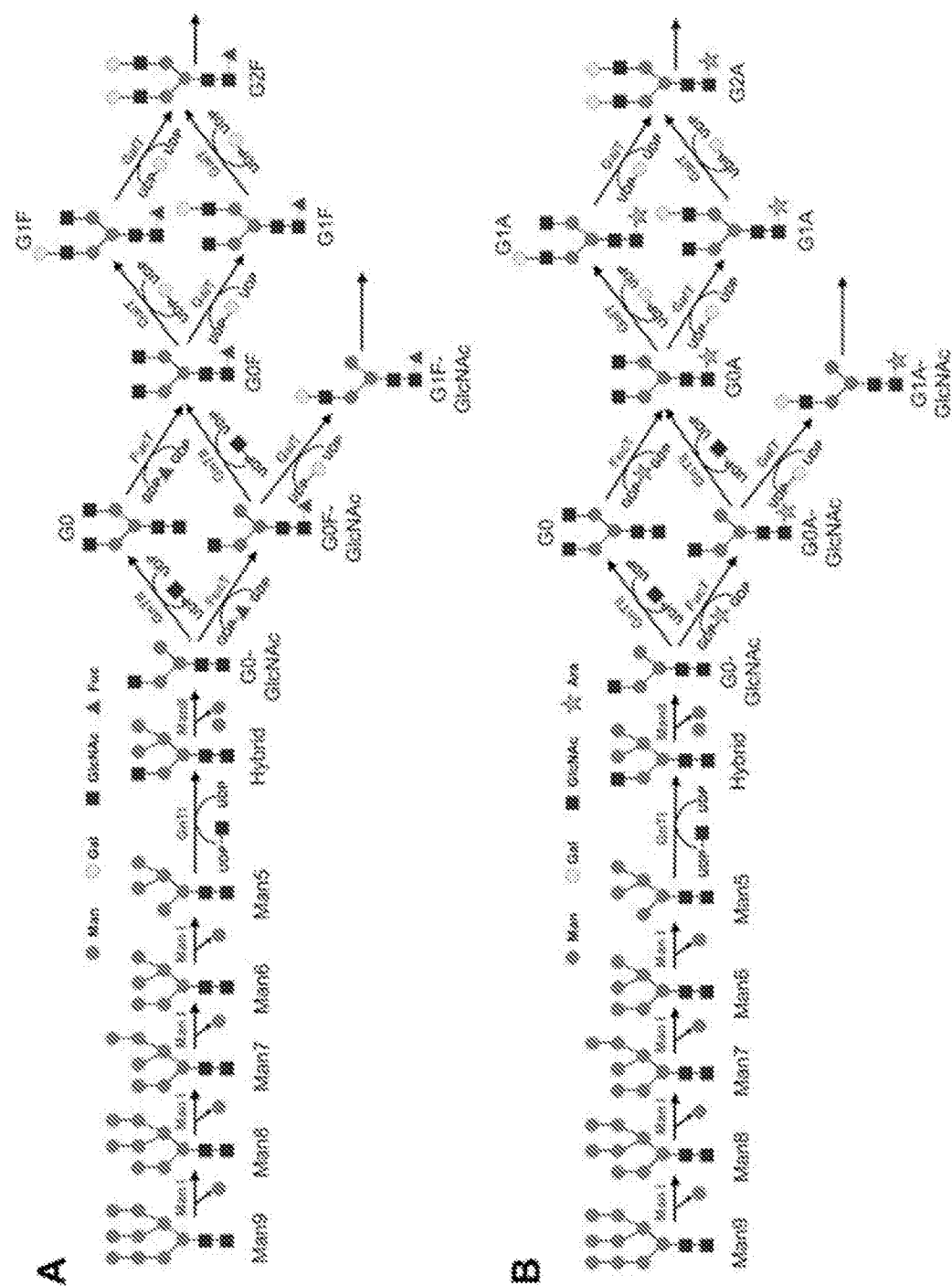
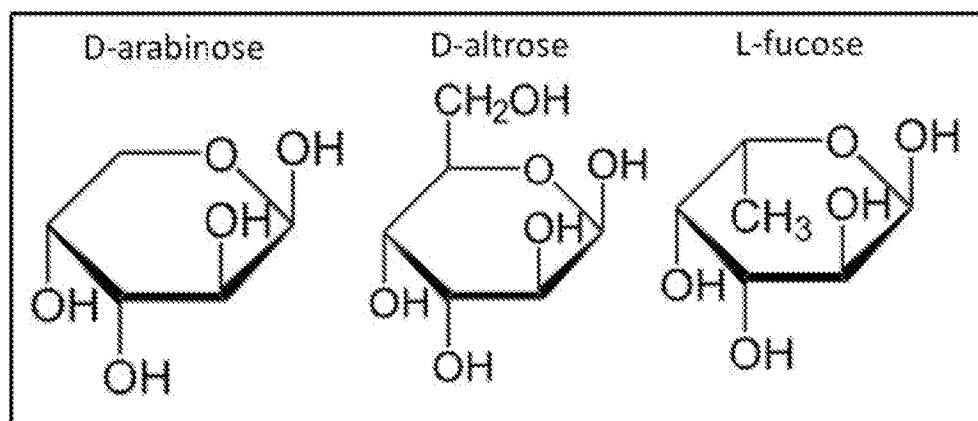


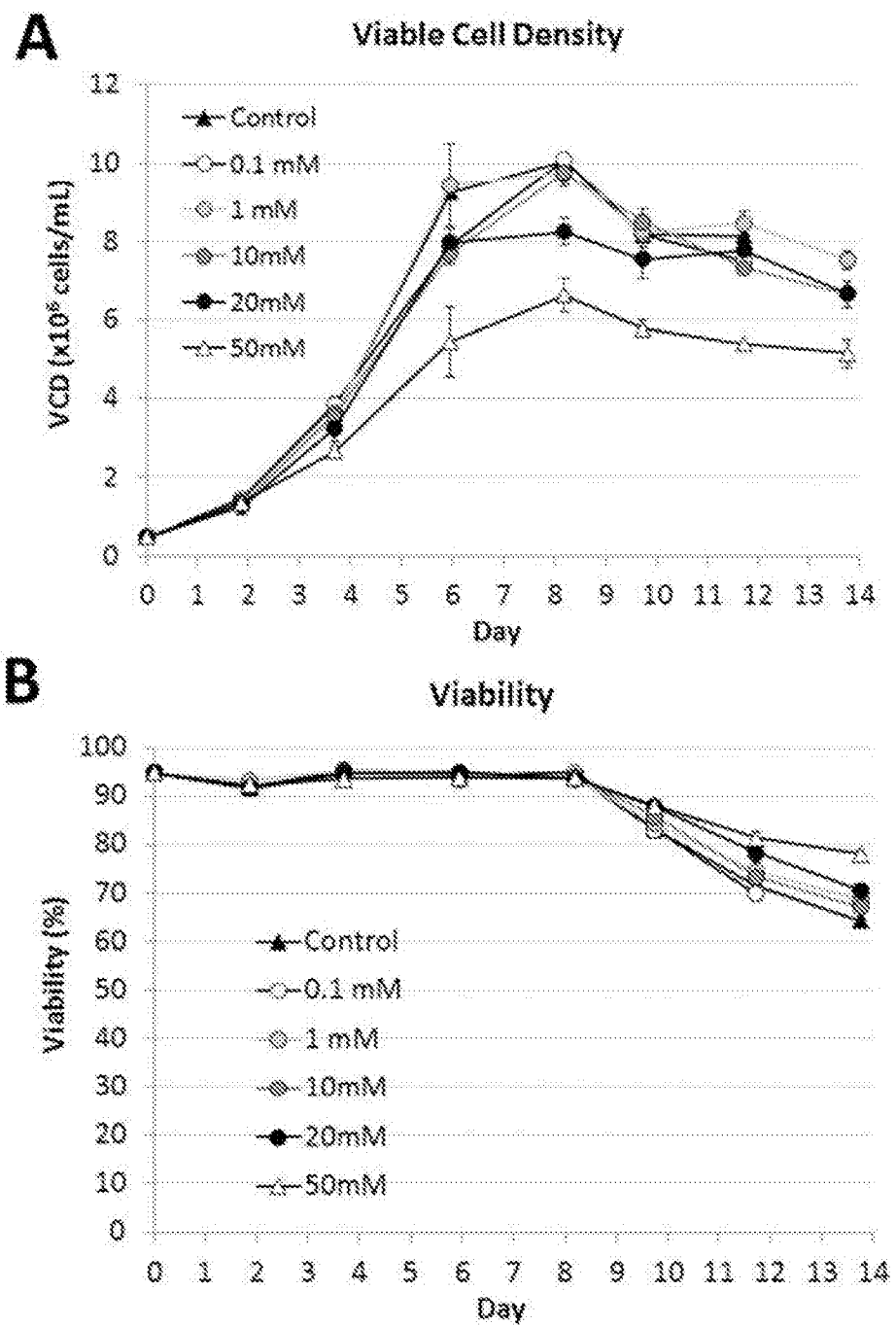
Figure 1

Figure 2



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Figure 3



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Figure 3 (cont).

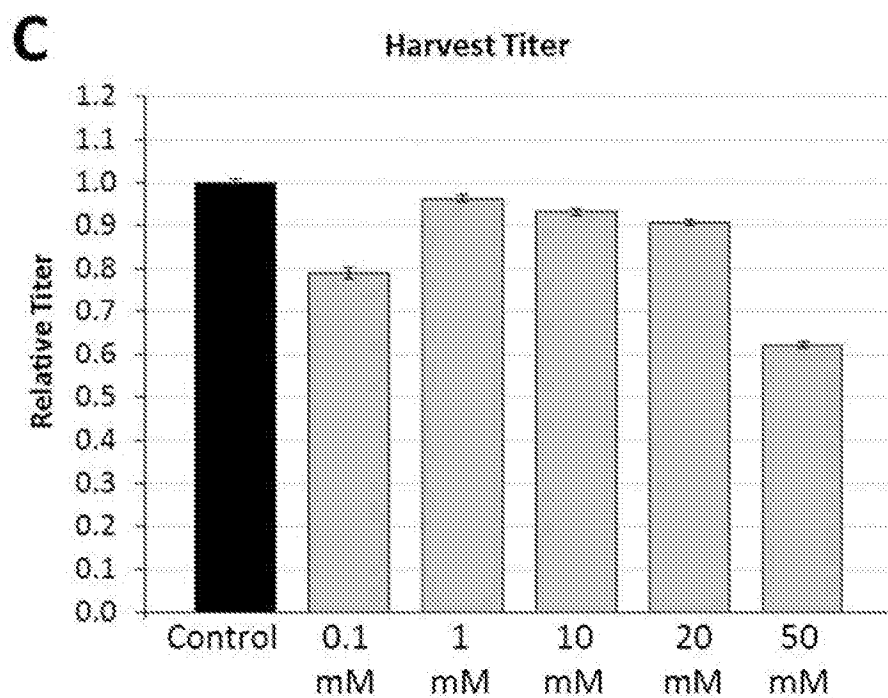
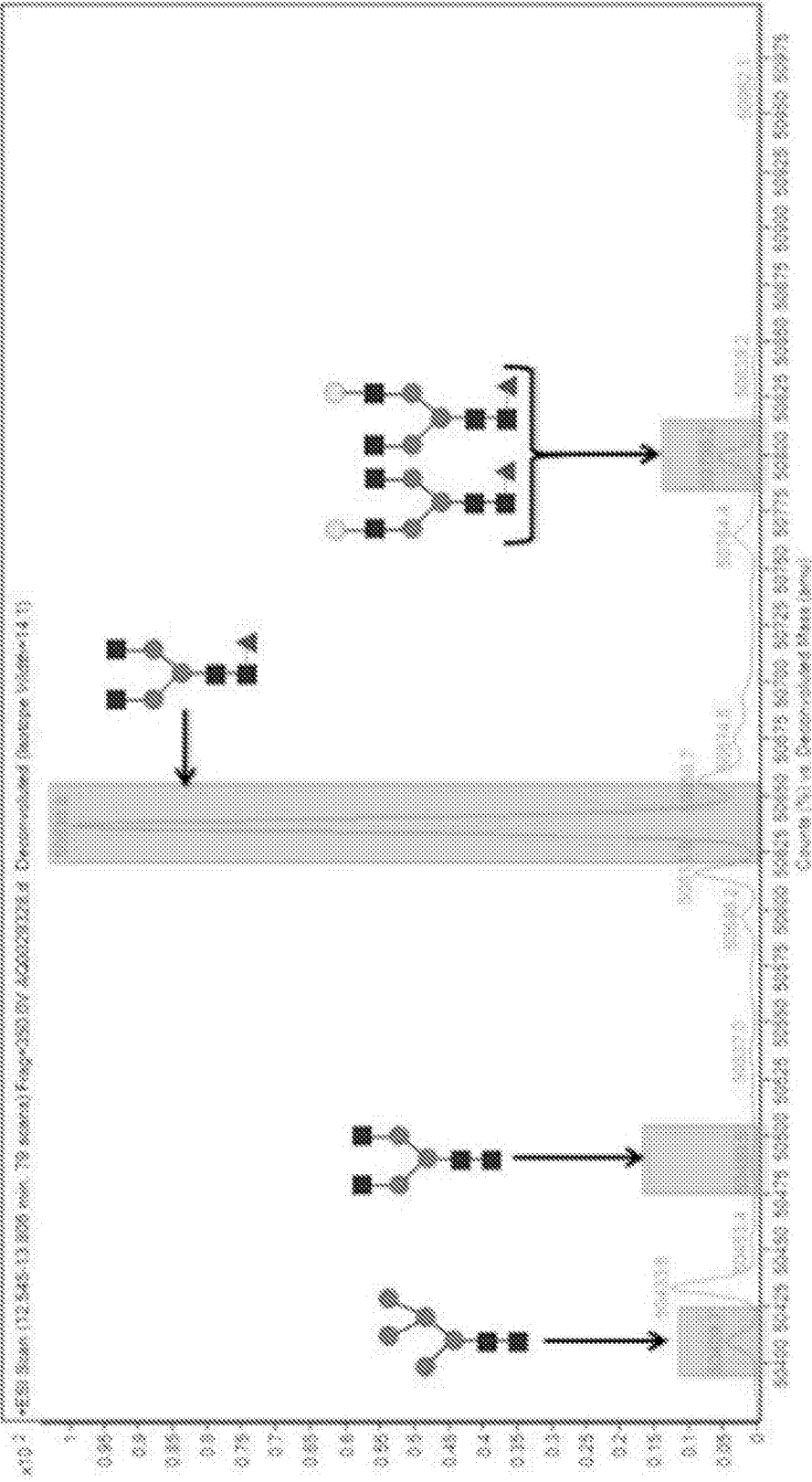


Figure 4
A



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Figure 4 (cont.)

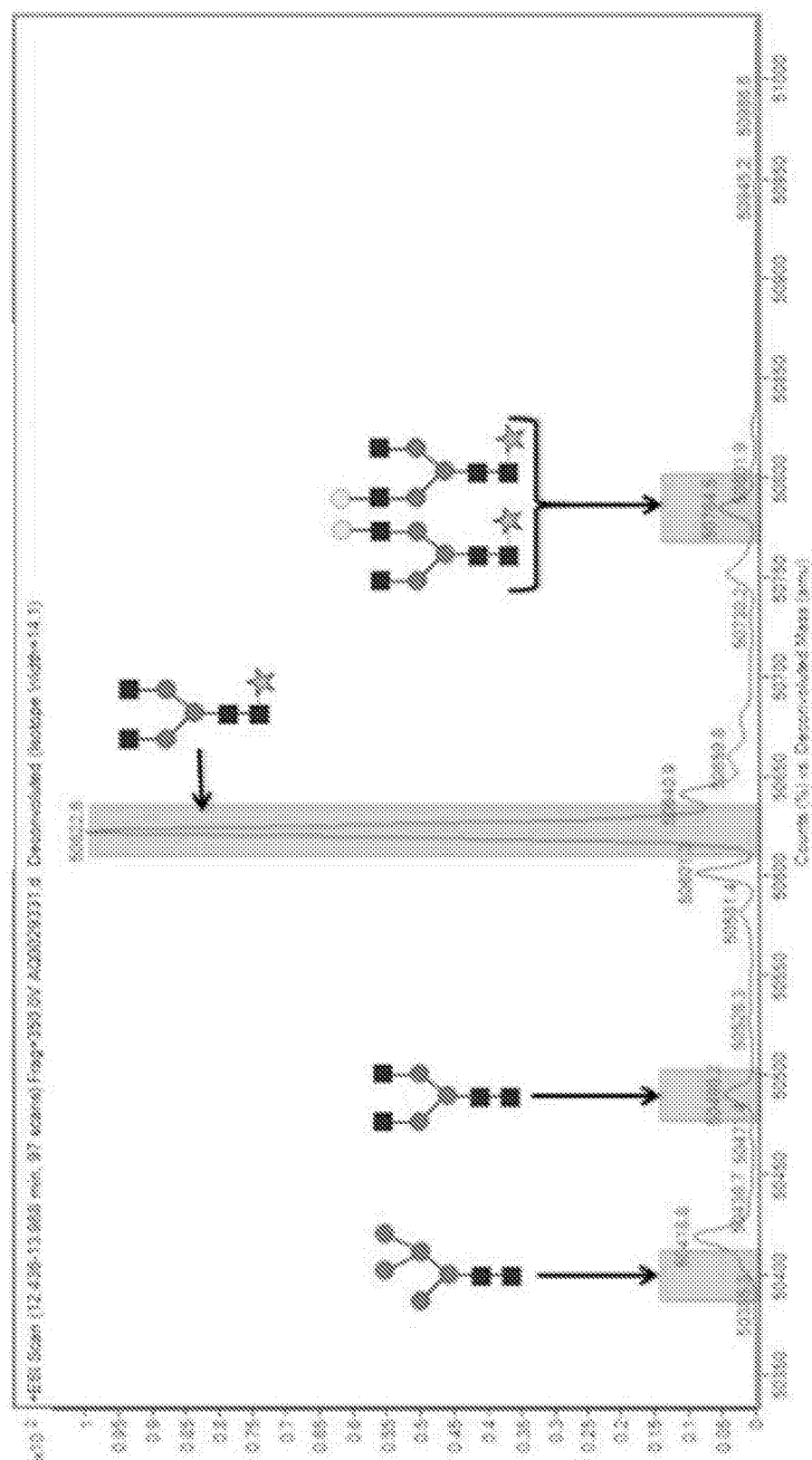


Figure 5

A

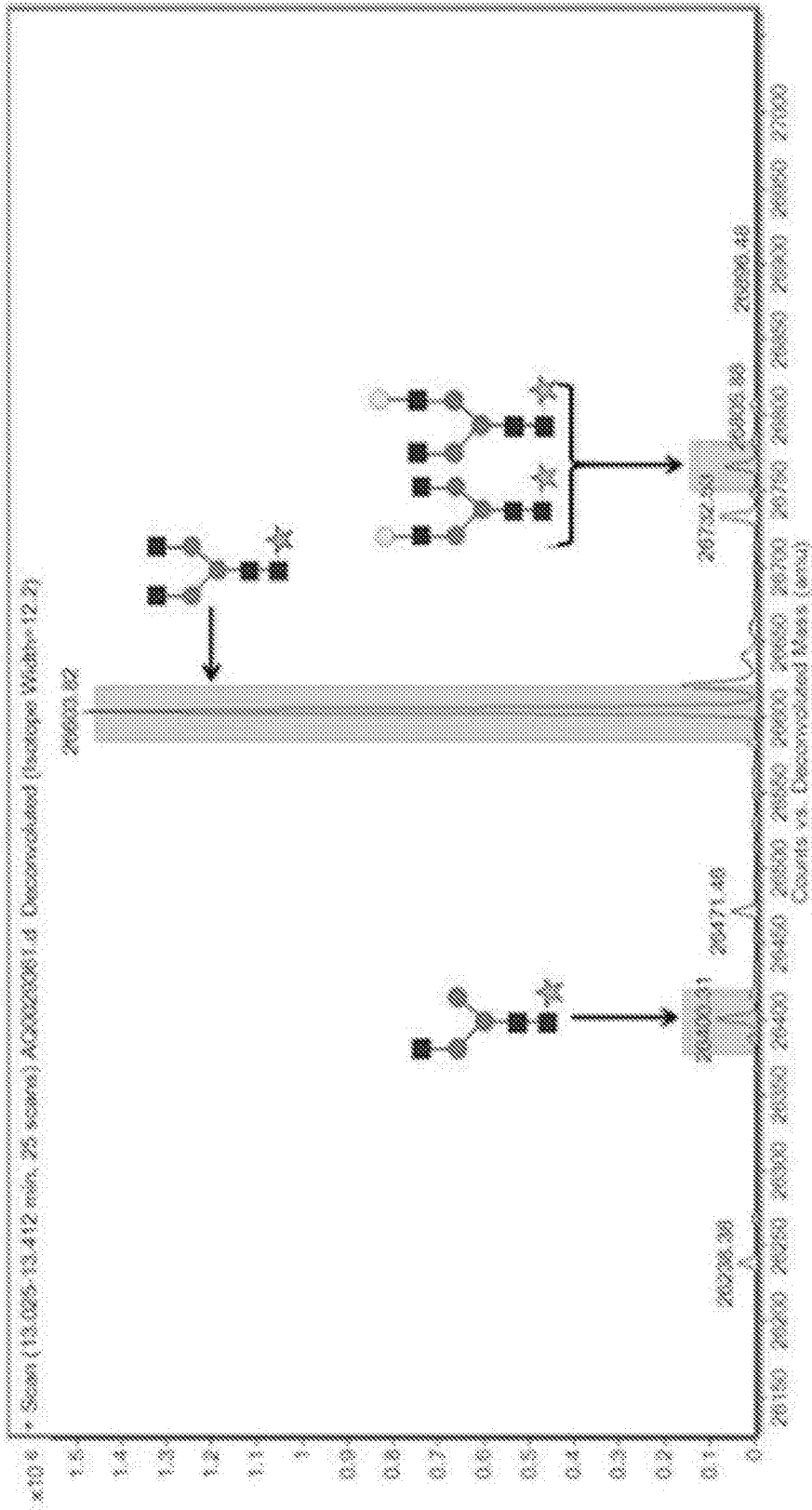
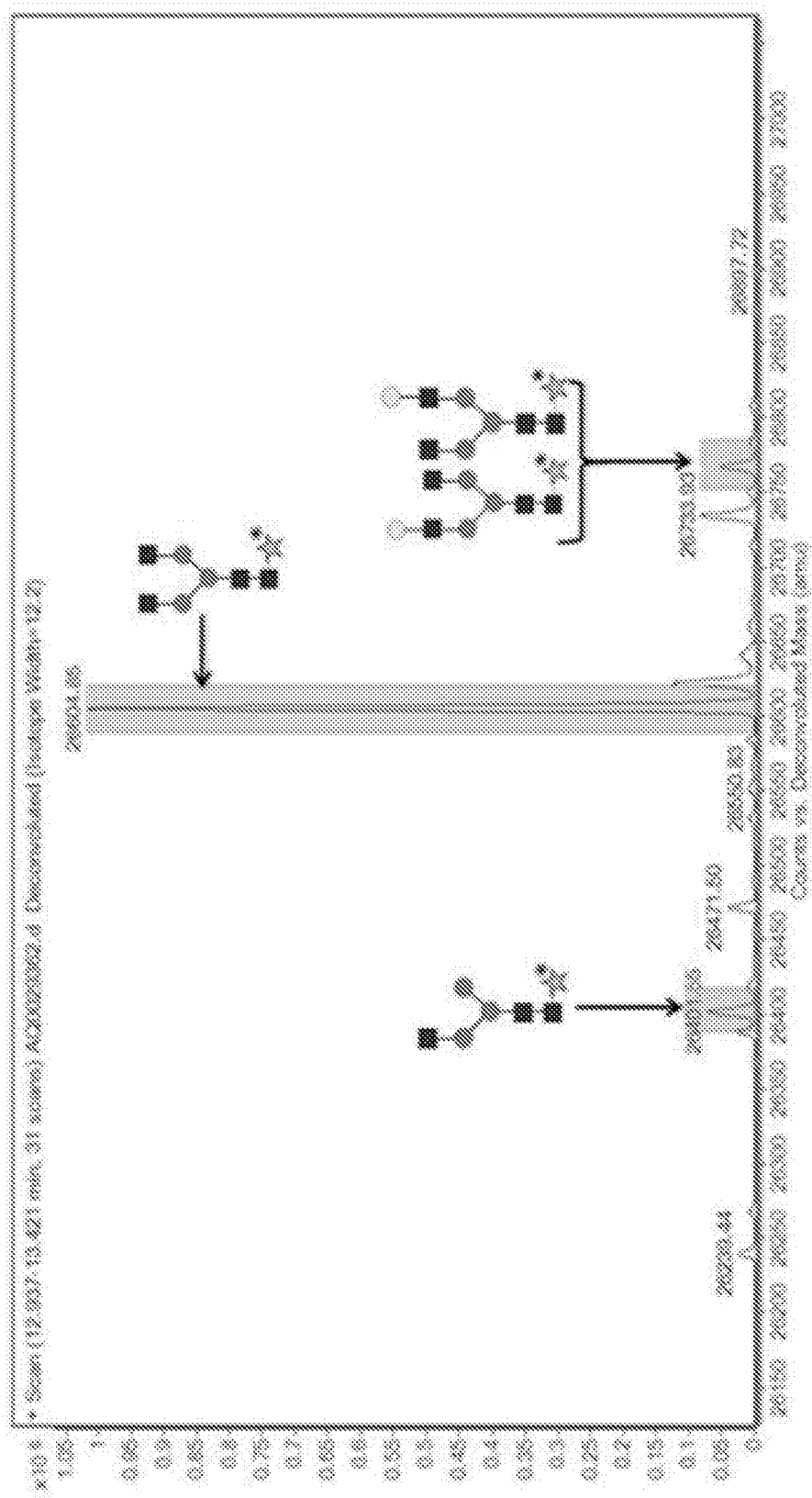
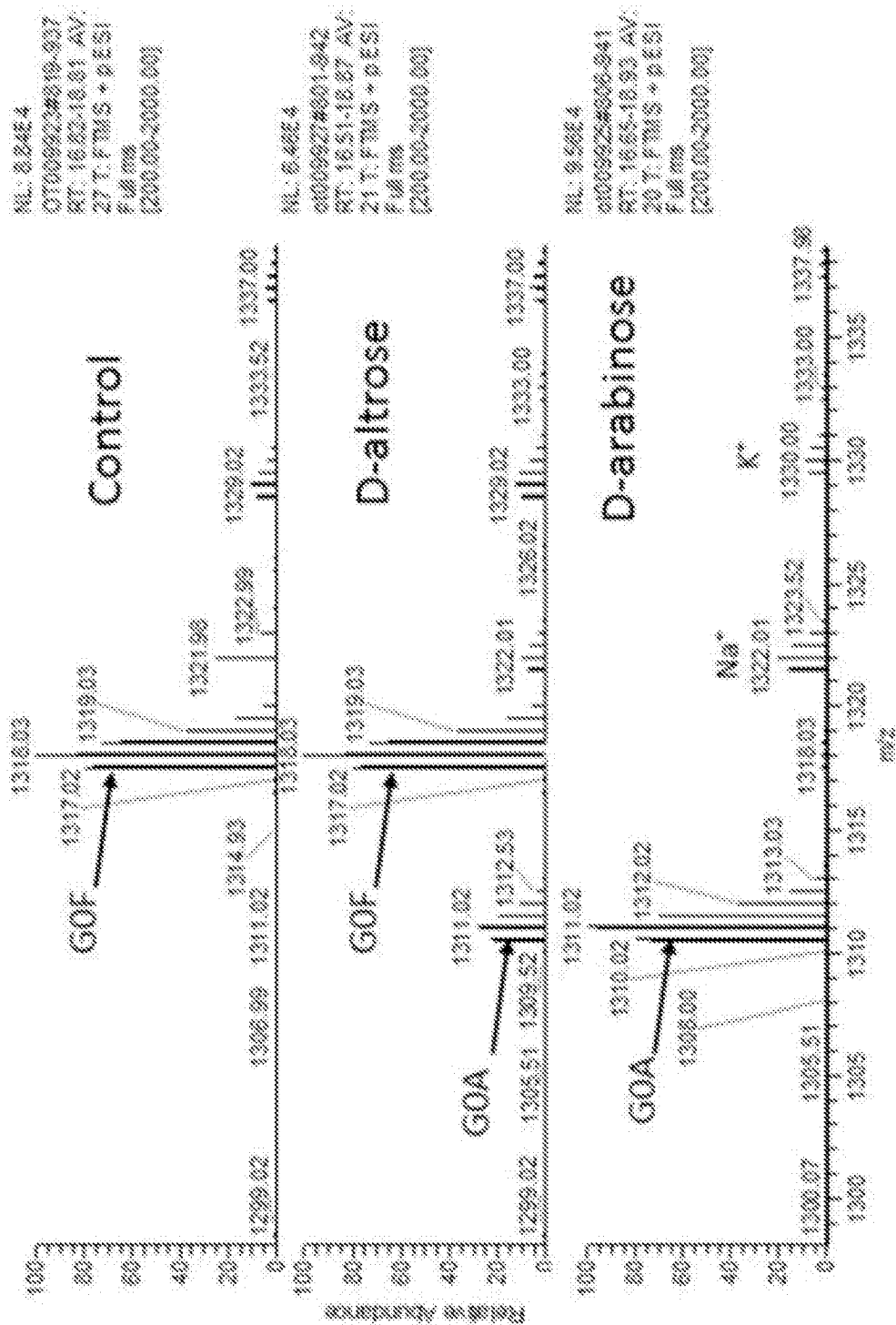


Figure 2 (cont.)



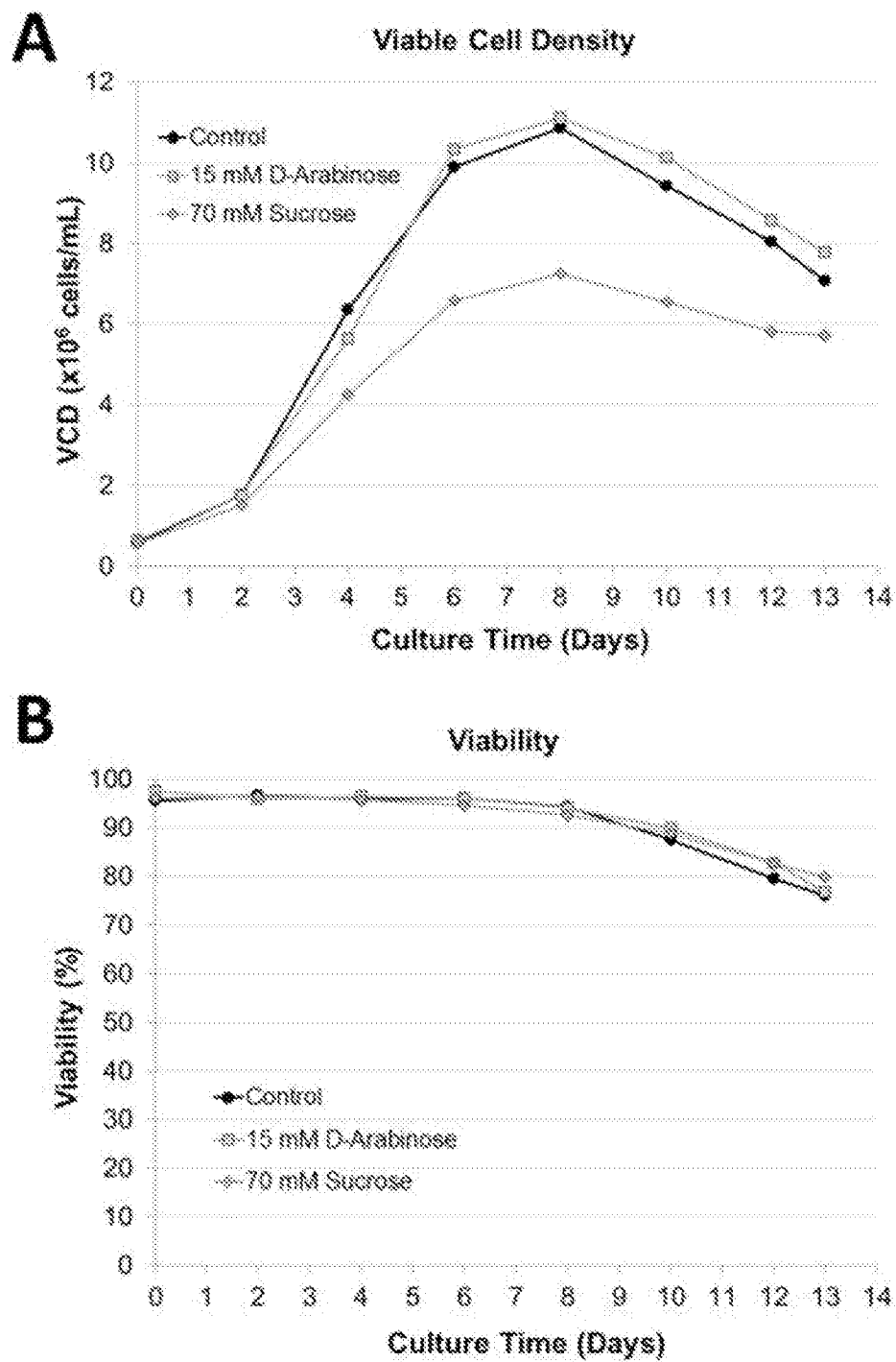
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Figure 6



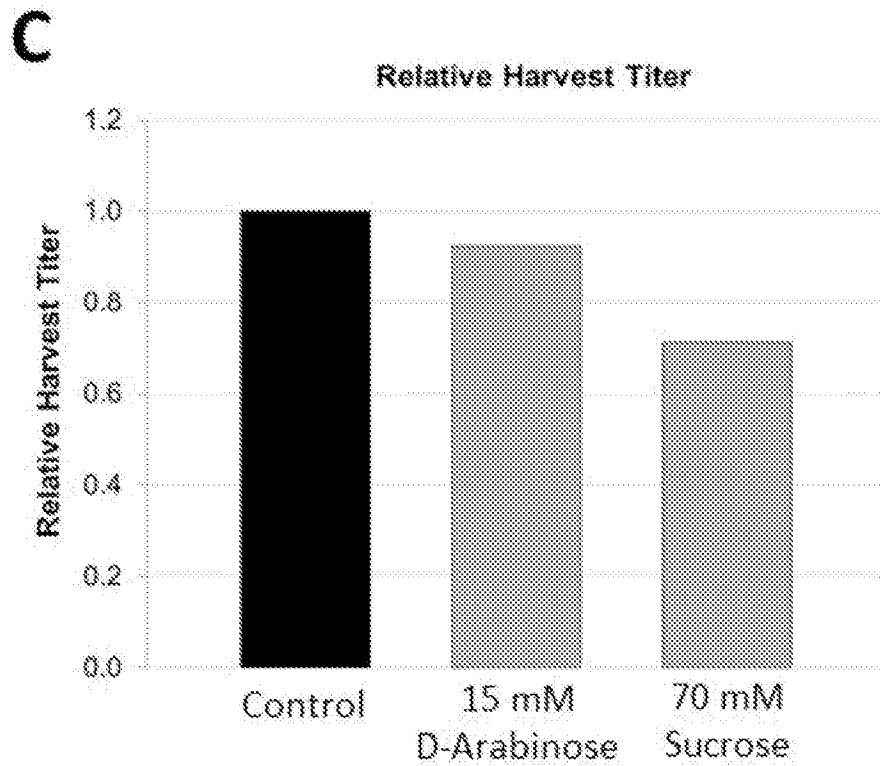
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Figure 7



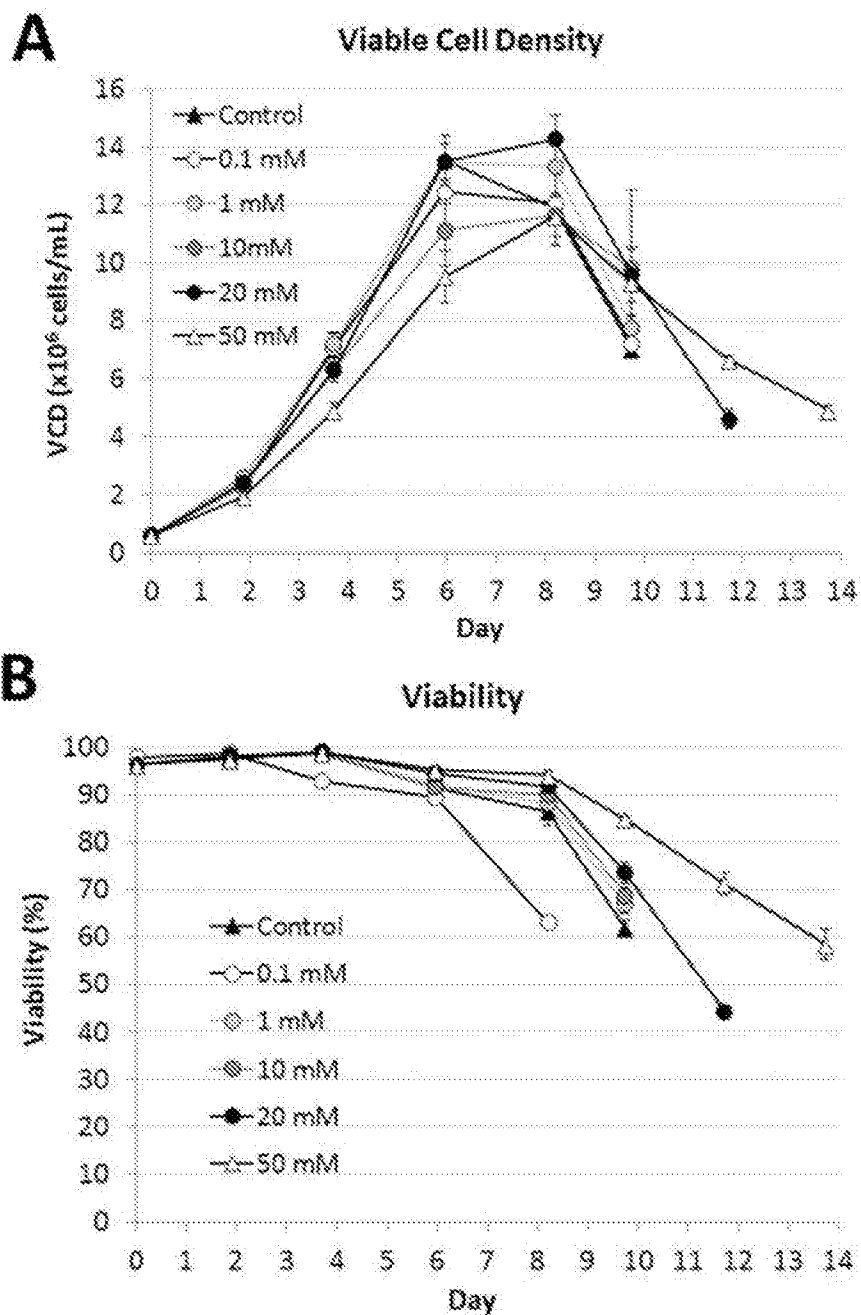
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Figure 7 (cont.)



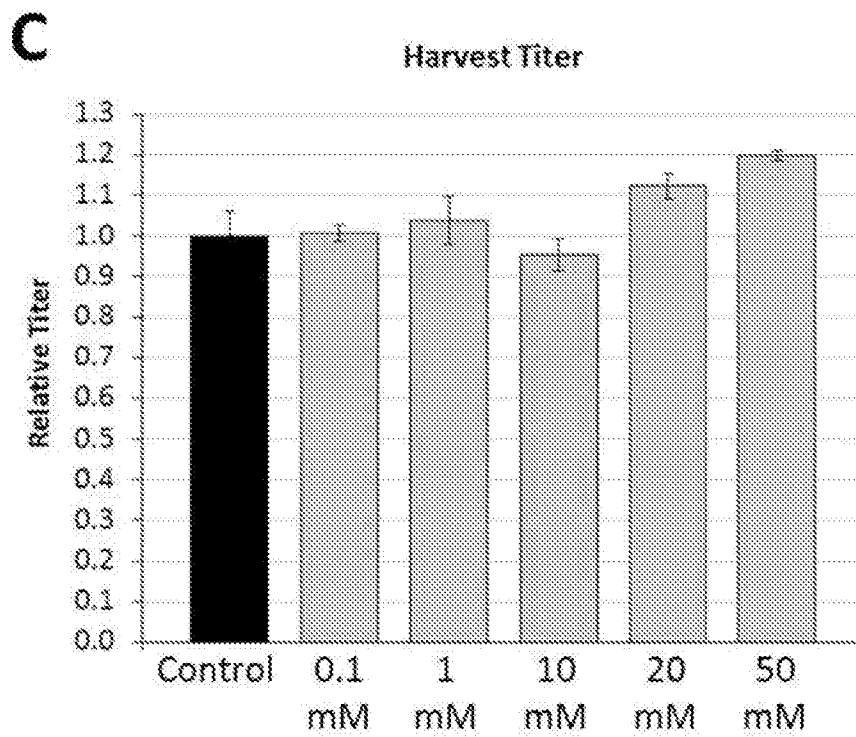
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Figure 8



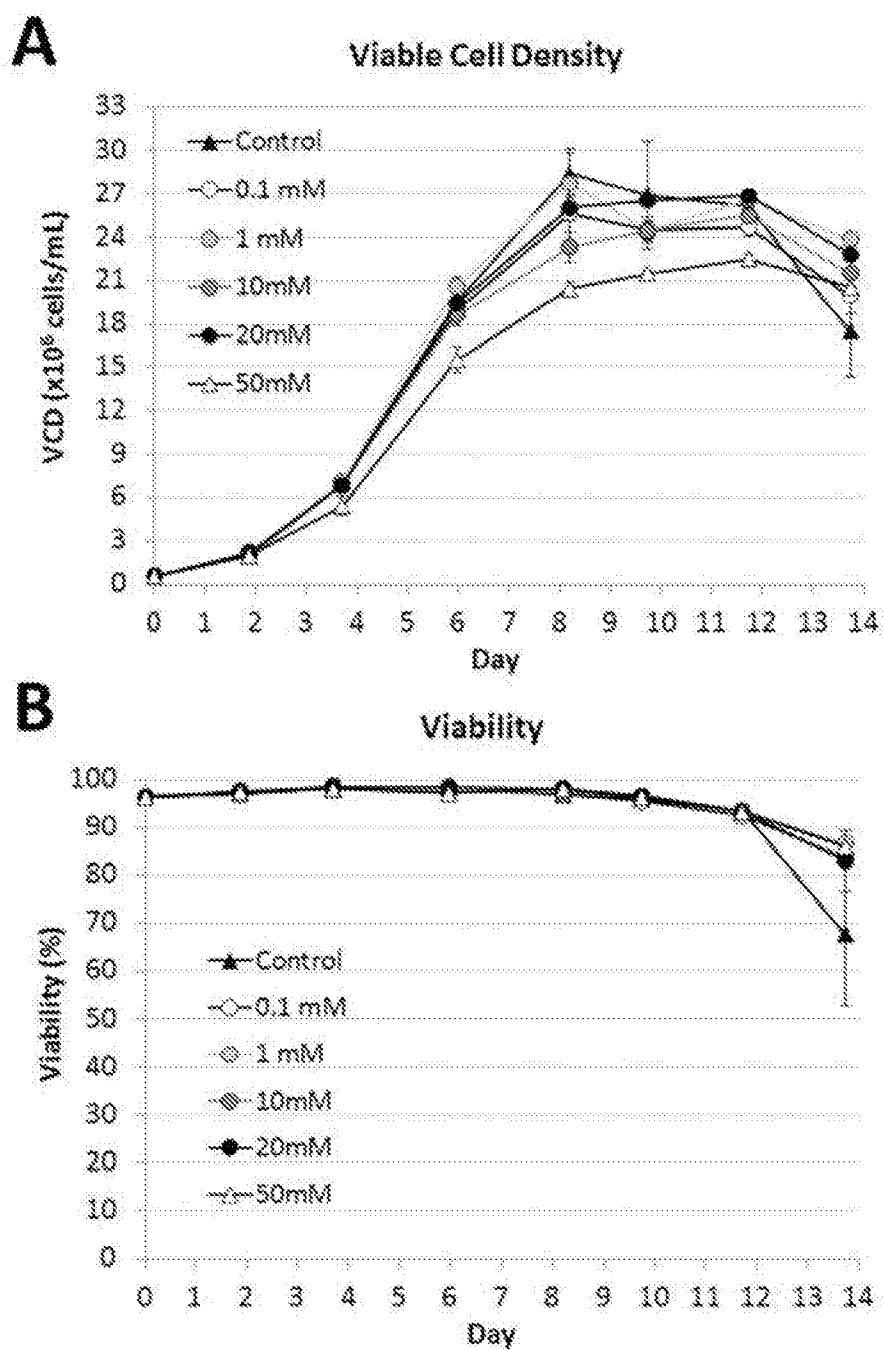
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Figure 8 (cont).



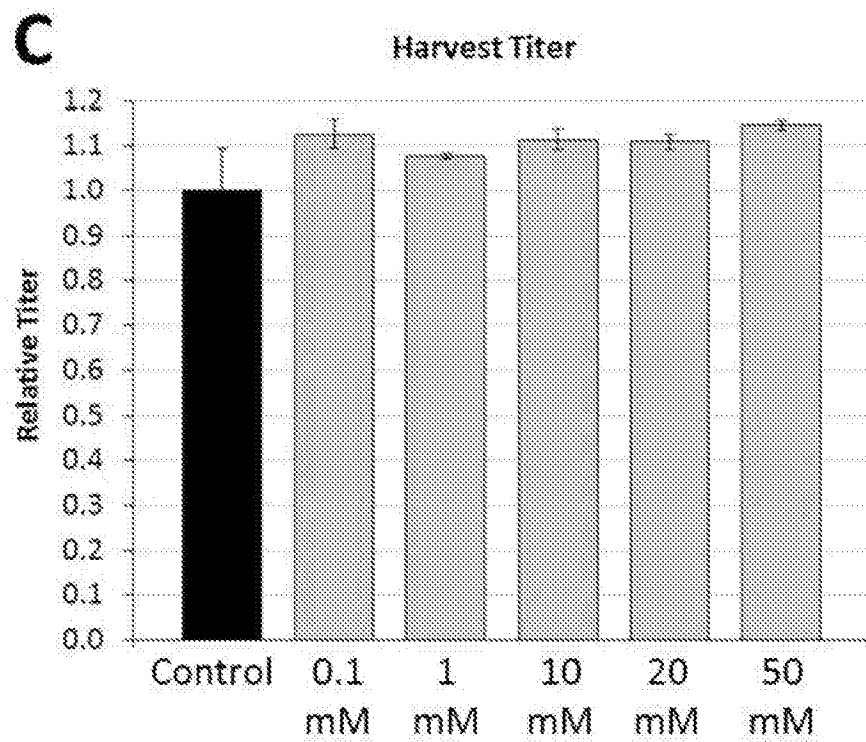
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Figure 9



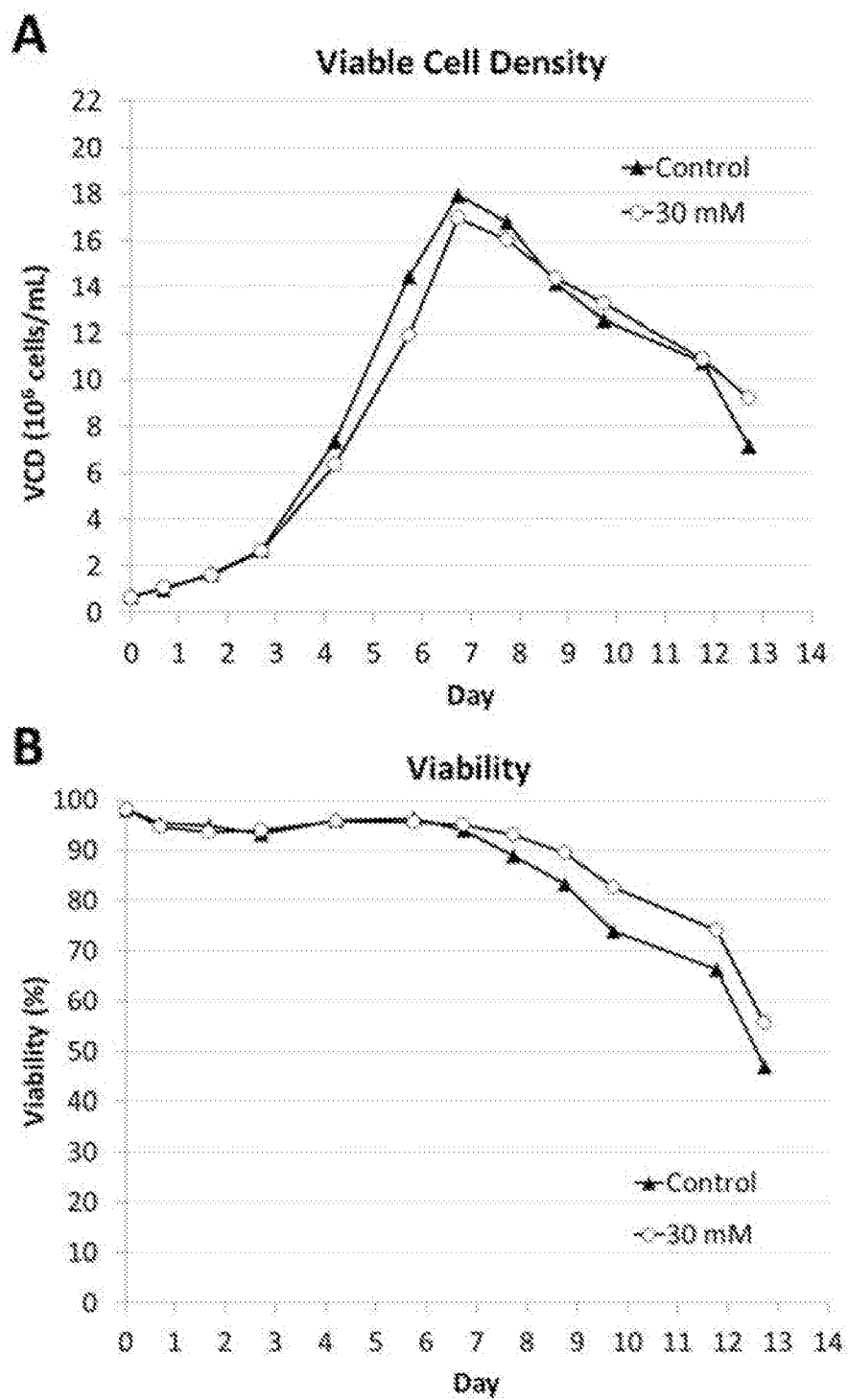
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Figure 9 (cont.)



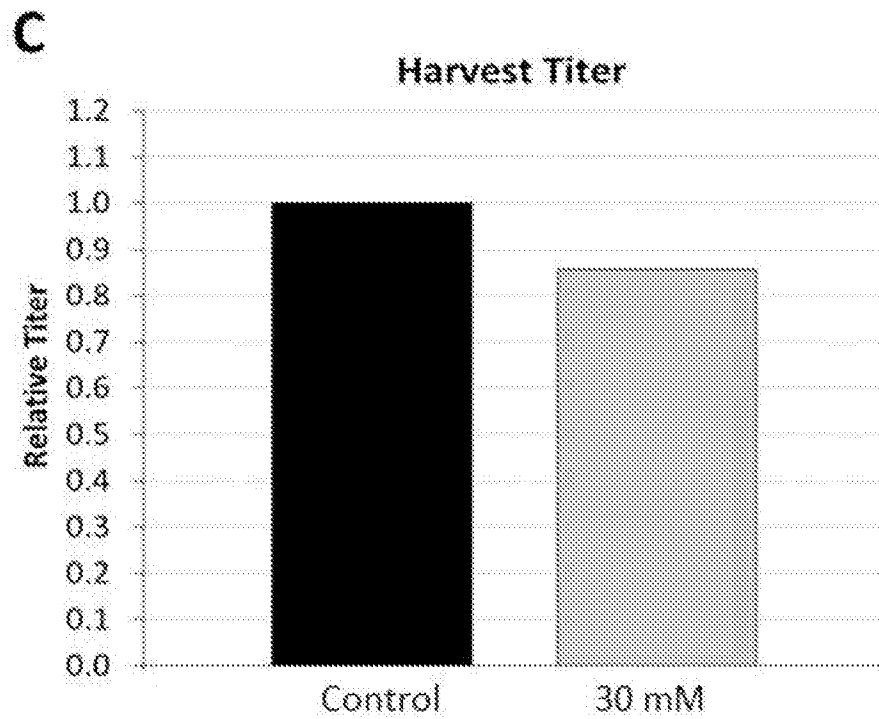
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Figure 10



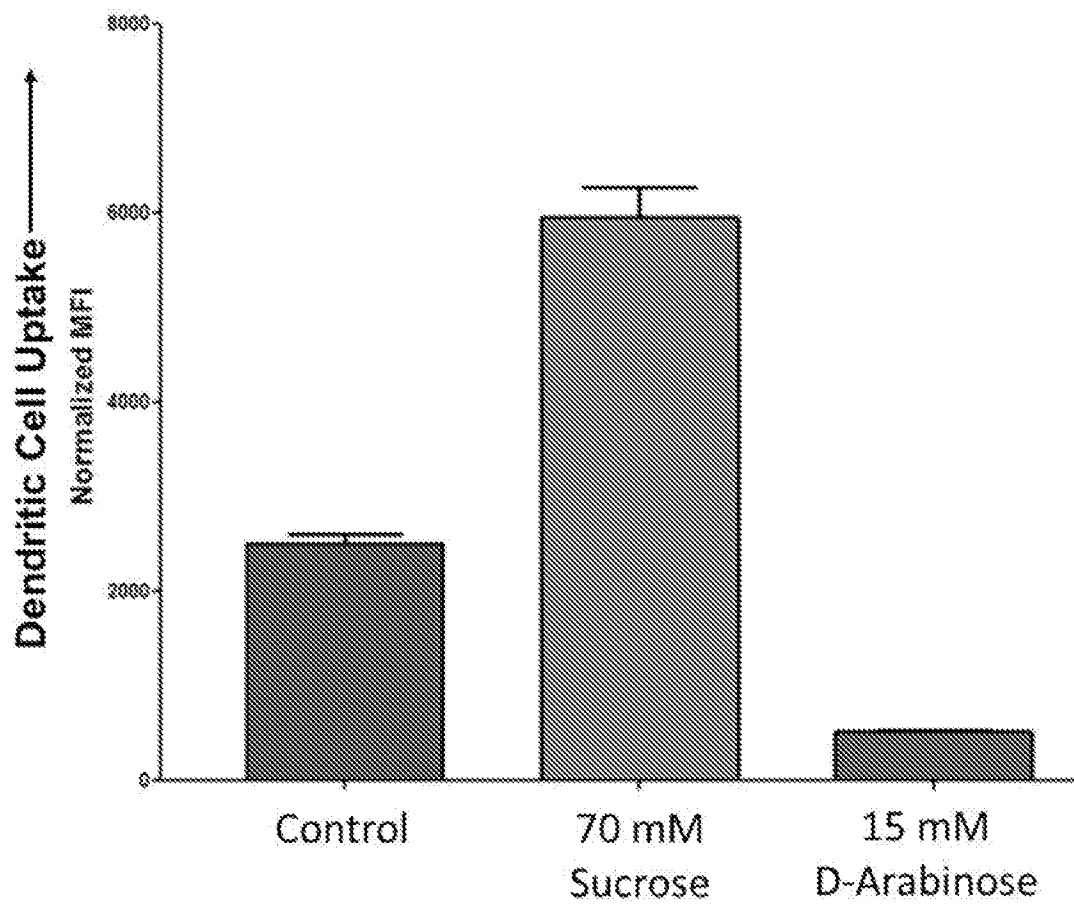
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Figure 10 (cont.)



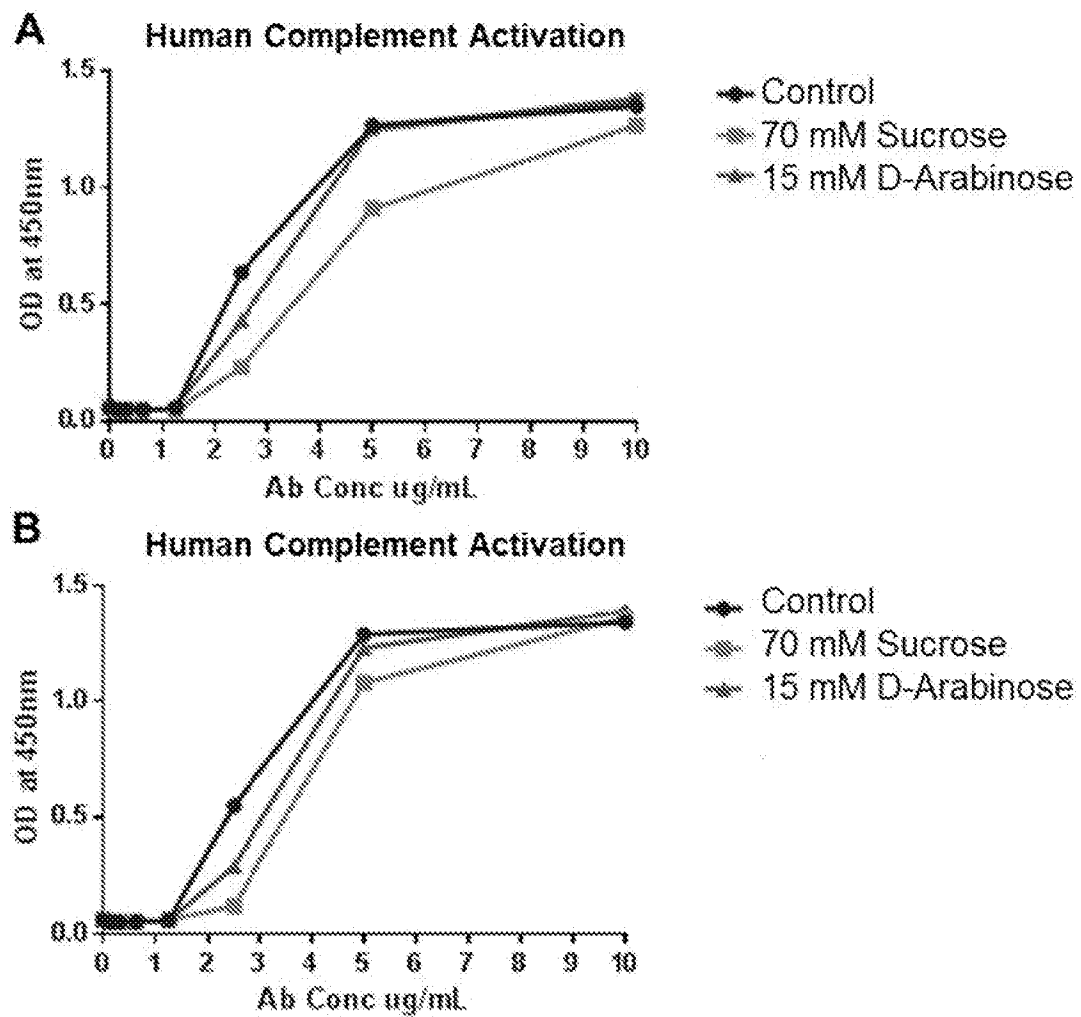
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Figure 11



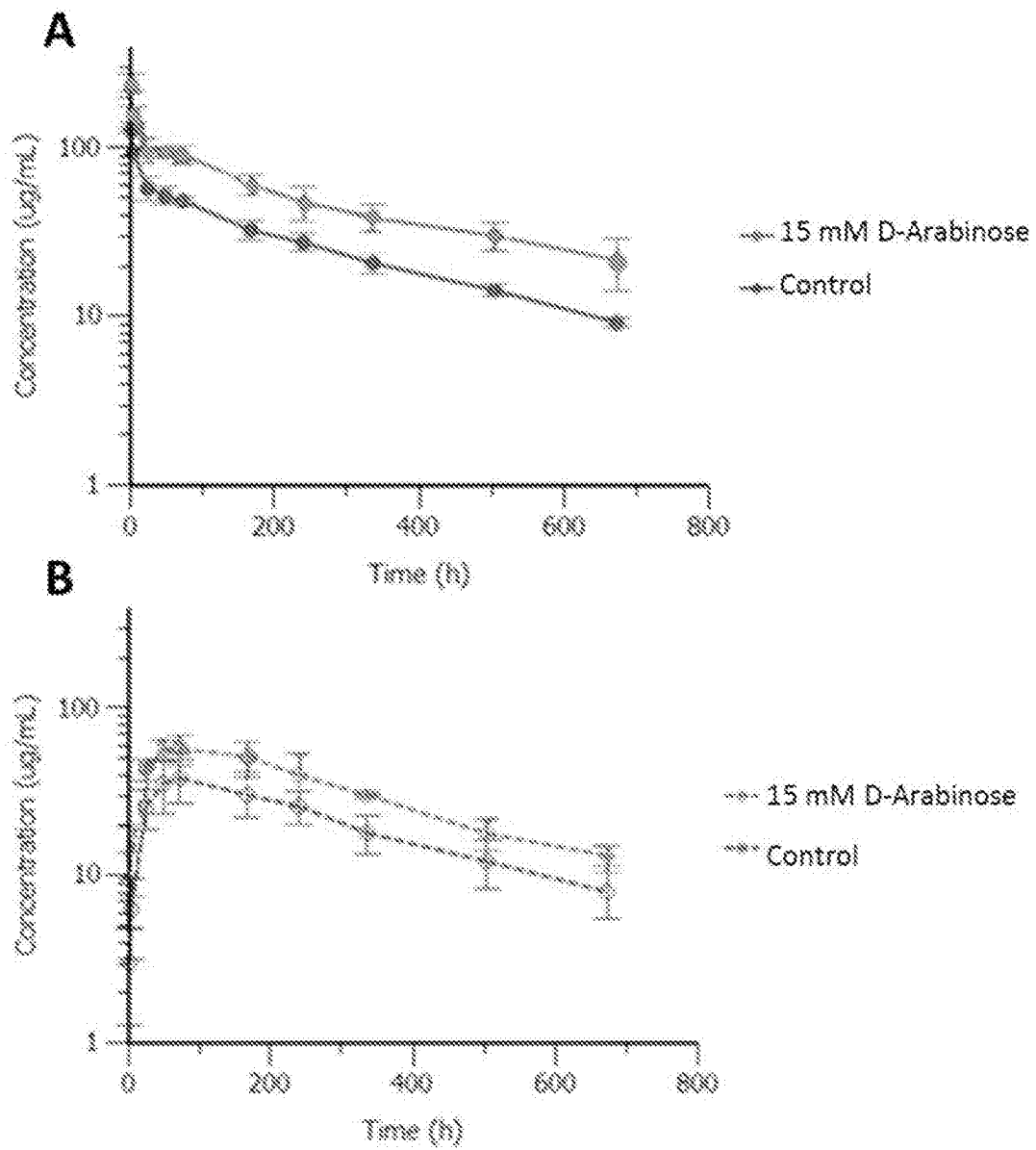
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Figure 12



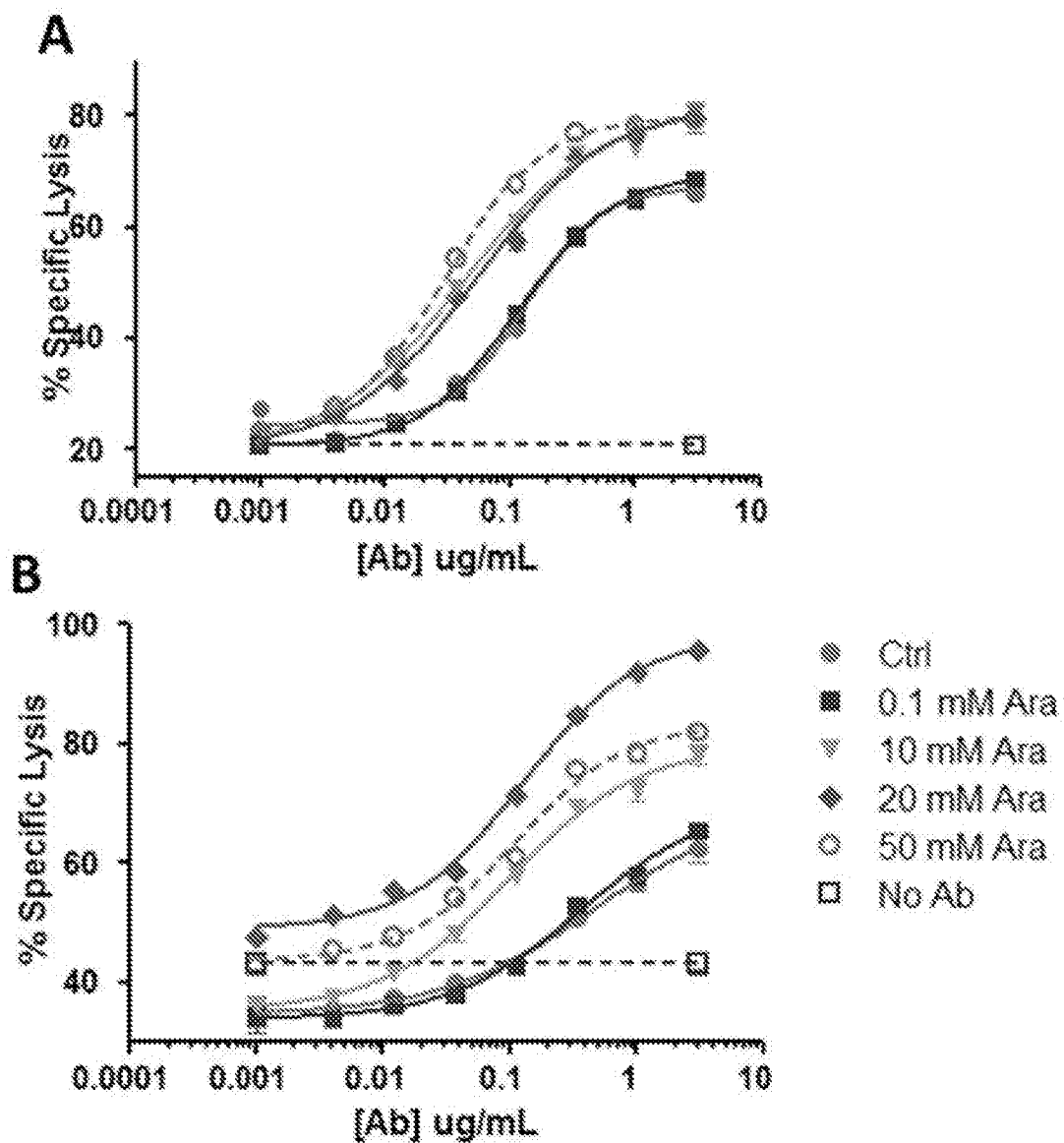
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Figure 13



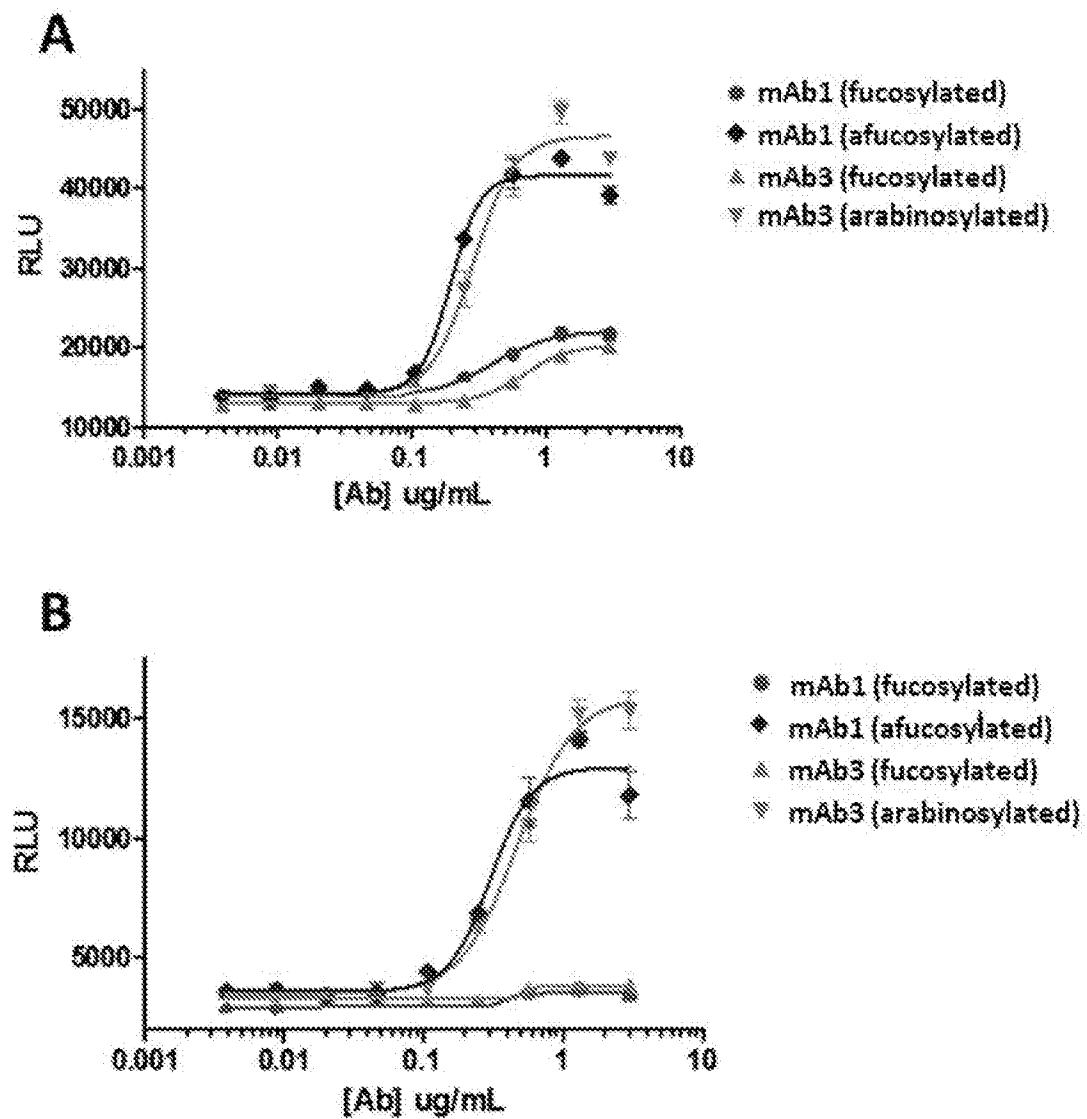
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Figure 14



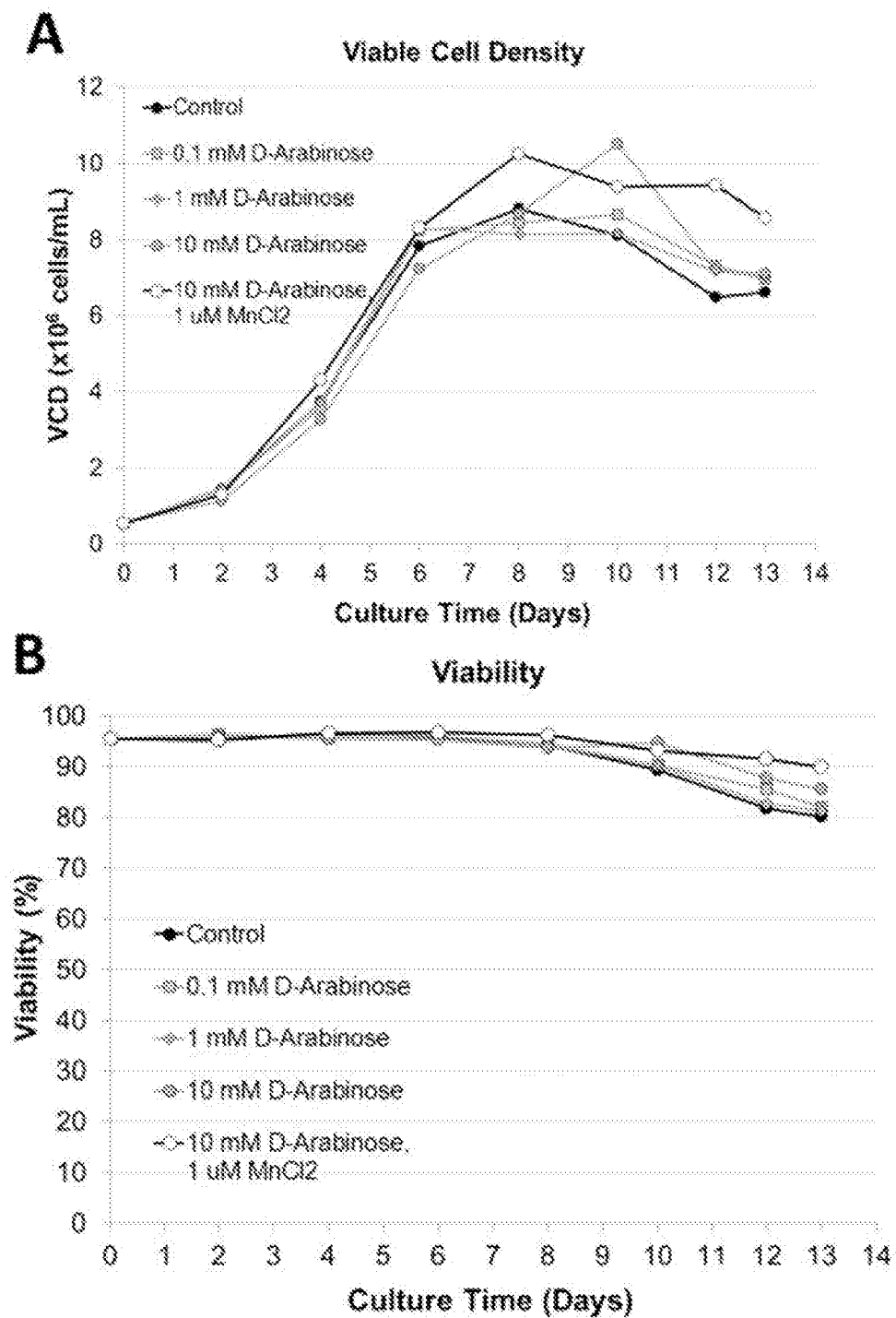
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Figure 15



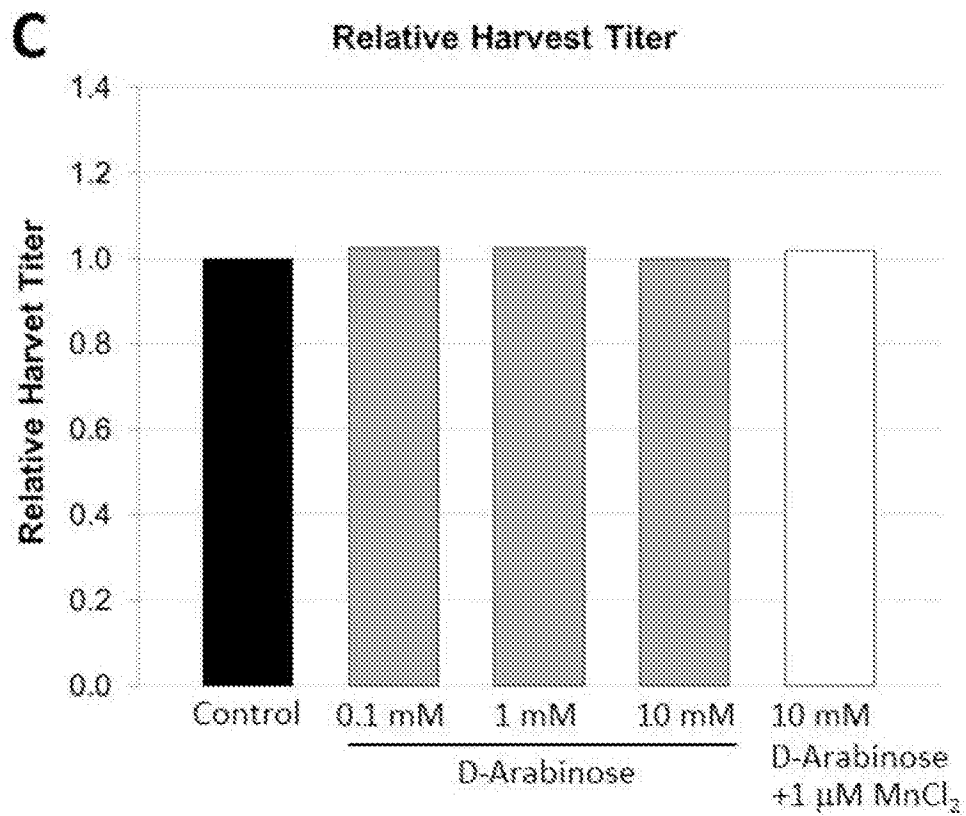
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Figure 16



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Figure 16 (cont.)



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/020931

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☐ forming part of the international application as filed:
- ☐ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☒ furnished subsequent to the international filing date for the purposes of international search only:
- ☒ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/020931

A. CLASSIFICATION OF SUBJECT MATTER INV. C07K16/00 C07K16/24 C07K16/28 C12P21/08 C12N5/02 ADD.											
According to International Patent Classification (IPC) or to both national classification and IPC											
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C07K C12P C12N											
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched											
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data, BIOSIS, EMBASE											
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>X</td> <td>JOJANNEKE H.J HUCK ET AL: "Evaluation of pentitol metabolism in mammalian tissues provides new insight into disorders of human sugar metabolism", MOLECULAR GENETICS AND METABOLISM, vol. 82, no. 3, 1 July 2004 (2004-07-01), pages 231-237, XP55276914, AMSTERDAM, NL</td> <td>1-35</td> </tr> <tr> <td>Y</td> <td>ISSN: 1096-7192, DOI: 10.1016/j.ymgme.2004.05.003 figure 2 page 232, last paragraph ----- -/--</td> <td>36-38, 51,52, 54-57</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X	JOJANNEKE H.J HUCK ET AL: "Evaluation of pentitol metabolism in mammalian tissues provides new insight into disorders of human sugar metabolism", MOLECULAR GENETICS AND METABOLISM, vol. 82, no. 3, 1 July 2004 (2004-07-01), pages 231-237, XP55276914, AMSTERDAM, NL	1-35	Y	ISSN: 1096-7192, DOI: 10.1016/j.ymgme.2004.05.003 figure 2 page 232, last paragraph ----- -/--	36-38, 51,52, 54-57
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.									
X	JOJANNEKE H.J HUCK ET AL: "Evaluation of pentitol metabolism in mammalian tissues provides new insight into disorders of human sugar metabolism", MOLECULAR GENETICS AND METABOLISM, vol. 82, no. 3, 1 July 2004 (2004-07-01), pages 231-237, XP55276914, AMSTERDAM, NL	1-35									
Y	ISSN: 1096-7192, DOI: 10.1016/j.ymgme.2004.05.003 figure 2 page 232, last paragraph ----- -/--	36-38, 51,52, 54-57									
☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.											
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Date of the actual completion of the international search		Date of mailing of the international search report									
2 June 2016		20/06/2016									
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer									
		Brouns, Gaby									

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International application No
PCT/US2016/020931

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JUNSUO S. LI ET AL: "Characterization of N-linked oligosaccharides in chorion peroxidase of Aedes aegypti mosquito", PROTEIN SCIENCE, vol. 14, no. 9, 1 September 2005 (2005-09-01), pages 2370-2386, XP055276408, US ISSN: 0961-8368, DOI: 10.1110/ps.051419105 table 1	1,2,10, 11,19,20
Y	----- Ole Hindsgaul ET AL: "Use of Glycosyltransferases in Synthesis of Unnatural Oligosaccharide Analogs" In: "Enzymes in Carbohydrate Synthesis", 24 June 1991 (1991-06-24), American Chemical Society, Washington, DC, XP055276715, ISBN: 978-0-8412-1322-7 vol. 466, pages 38-50, DOI: 10.1021/bk-1991-0466.ch003, page 40, paragraph 4 figure 3	36-38, 51,52, 54-57
A	----- SHIELDS R L ET AL: "Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human FcγRIII and antibody-dependent cellular toxicity", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 277, no. 30, 26 July 2002 (2002-07-26), pages 26733-26740, XP002964542, ISSN: 0021-9258, DOI: 10.1074/JBC.M202069200 cited in the application	1-60
A	----- RYAN HARYADI ET AL: "CHO-gmt5, a novel CHO glycosylation mutant for producing afucosylated and asialylated recombinant antibodies", BIOENGINEERED, vol. 4, no. 2, 1 March 2013 (2013-03-01), pages 90-94, XP055238669, US ISSN: 2165-5979, DOI: 10.4161/bioe.22262 ----- -/--	1-60

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/020931

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HOSSLER P ET AL: "Cell culture media supplementation of uncommonly used sugars sucrose and tagatose for the targeted shifting of protein glycosylation profiles of recombinant protein therapeutics", BIOTECHNOLOGY PROGRESS, AMERICAN INSTITUTE OF CHEMICAL ENGINEERS, US, vol. 30, no. 6, 1 November 2014 (2014-11-01), pages 1419-1431, XP002754745, ISSN: 8756-7938, DOI: 10.1002/BTPR.1968 [retrieved on 2014-08-19] cited in the application -----	1-60
A	ARND STURM: "Heterogeneity of the complex N-linked oligosaccharides at specific glycosylation sites of two secreted carrot glycoproteins", EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 199, no. 1, 1 July 1991 (1991-07-01), pages 169-179, XP055276404, GB ISSN: 0014-2956, DOI: 10.1111/j.1432-1033.1991.tb16106.x figures 6, 15 -----	1-35
A	Bernard Priem ET AL: "Structure of Ten Free N-Clycans in Ripening Tomato Fruit' Arabinose 1s a Constituent of a Plant N-Glycan", Plant Physiol, 1 January 1993 (1993-01-01), pages 445-458, XP055276583, Retrieved from the Internet: URL:http://www.plantphysiol.org/content/102/2/445.full.pdf#page=1&view=FitH figure 4 -----	1-35