

CLAIMS

1. A substantially purified neutral active PH20 glycoprotein, ~~that wherein~~
the glycoprotein consists of:

a) a polypeptide that consists of a sequence of amino acid residues that:

5 (i) is truncated at a C-terminal residue within 10 amino acids before or at
 of amino acid 483 in the sequence of amino acids set forth in SEQ ID NO:1, or

(ii) consists of a sequence of amino acid residues having at least 98%
91%-sequence identity to the sequence of amino acids that consists of residues
36-483 of SEQ ID NO:1 thereto; and

10 b) ~~contains~~ at least one N-linked sugar moiety, wherein the N-linked sugar
 moiety is covalently attached to an asparagine residue of the polypeptide.

2. The glycoprotein of claim 1, wherein the polypeptide is selected from:

15 (a) a polypeptide that ~~comprises~~ consists of a sequence of amino acids ~~that~~
~~includes having~~ at least about 98% 91%-amino acid sequence identity with the sequence
 of amino acids that consists of residues 36-483 ~~1-483~~ as set forth in SEQ ID NO. 1; and

(b) a polypeptide that ~~comprises~~ consists of a sequence of amino acids encoded
 by the sequence of nucleotides 1-1449 as set forth in SEQ ID NO. 6.

20 3. The glycoprotein of claim 1, wherein the polypeptide consists of a
sequence of amino acid residues having at least 98% sequence identity to the sequence
of amino acids that consists of residues 36-483 of SEQ ID NO:1.

4. The glycoprotein of claim 1, wherein said sugar moiety is covalently attached to an asparagine residue selected from amino acids 82, 166, 235, 254, 368, or 393 as set forth in SEQ ID NO. 1.

5 5. A substantially purified neutral active PH20 glycoprotein, consisting of:

a) a polypeptide consisting of the sequence of amino acid residues set forth as 1-459 in SEQ ID NO:3; and

b) at least one N-linked sugar moiety, wherein the N-linked sugar moiety is covalently attached to an asparagine residue of the polypeptide, wherein

10 the glycoprotein is catalytically active; and

the glycoprotein is secreted.

64. The glycoprotein of claim 1, wherein said sugar moiety is covalently linked to said polypeptide through a PNGase sensitive bond.

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75. The glycoprotein of claim 1, wherein said sugar moiety is a high mannose type.

86. The glycoprotein of claim 1, wherein said sugar moiety is a complex
20 type.

97. The glycoprotein of claim 1, wherein said sugar moiety is a hybrid type.

108. The glycoprotein of claim 1, wherein said sugar moiety further comprises at least one terminated with sialic acid.

119. The glycoprotein of claim 108, wherein the polypeptide is super-sialated.

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~~10. The glycoprotein of claim 9, wherein the polypeptide comprises the sequence of amino acids set forth as amino acids 1-450 of SEQ ID NO. 3, amino acids 1-438 of SEQ ID NO. 3 or amino acids 1-459 of SEQ ID NO. 3.~~

10 1244. The glycoprotein of claim 19, wherein the polypeptide is truncated at an amino acid residue selected from among 477, 478, 479, 480, 481, 482 and 483.

1312. The glycoprotein of claim 1-A substantially purified neutral active PH20 glycoprotein that consists of: ~~a sequence of amino acids selected from among~~

15 a) a polypeptide that consists of:

(i) a sequence of amino acids residues set forth as 36-477, 36-478, 36-479, 36-480, 36-481, 36-482 or 36-483 of SEQ ID NO:1; or

(ii) a sequence of amino acids that contains amino acid substitutions in the sequence of amino acids set forth as 36-477, 36-478, 36-479, 36-480, 36-481, 36-482 or 36-483 of SEQ ID NO:1, whereby the amino-substituted hyaluronidase-glycoprotein-polypeptide consists of a sequence of amino acids that has at least 91% amino acid sequence identity to a sequence of amino acids set forth as amino acid residues 1-477, 1-478, 1-479, 1-480, 1-481, 1-482 or 1-483 ~~36-477, 36-478, 36-479, 36-480, 36-481, 36-482 or 36-483~~ of SEQ ID NO:1; and

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a portion thereof that includes amino acids 36-464 or amino-substituted residues thereof having 91% sequence identity therewith.

b) at least one N-linked sugar moiety, wherein the N-linked sugar moiety is covalently attached to an asparagine residue of the polypeptide.

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1413. The glycoprotein of claim 1 that consists of a sequence of amino acids set forth as amino acids 36-482 of SEQ ID NO:1.

1514. The glycoprotein of claim 1, wherein the glycoprotein is a human
10 glycoprotein.

1615. The glycoprotein of claim 1, wherein the polypeptide is a polypeptide as set forth in SEQ ID NO. 4.

15 1716. The glycoprotein of claim 1, with the proviso that the polypeptide does not comprise the complete sequence set forth in SEQ ID NO. 1 and includes at least amino acids 1-429 of SEQ ID NO. 4.

17. ~~The glycoprotein of claim 16, wherein up to about 10% of the amino-~~
20 ~~acids of said polypeptide are replaced with another amino acid.~~

18. ~~The glycoprotein of claim 16 wherein the resulting polypeptide has-~~
catalytic activity of at least 50 % of the unmutated polypeptide.

1819. ~~A~~The glycoprotein of claim 1, wherein the polypeptide is encoded by a nucleic acid molecule that encodes amino acids 36-482 of SEQ ID NO:1 or amino acids 1-482 of SEQ ID NO:1.

5 1920. The glycoprotein of claim 1819, wherein the polypeptide is secreted in CHO cells.

2021. The glycoprotein of claim 1, wherein the potency of the glycoprotein is greater than 40,000 USP Units/mg protein.

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2122. The glycoprotein of claim 2021, wherein the ~~hyaluronidase-glycoprotein~~ is sialated.

2223. The glycoprotein of claim 2021, wherein the potency is greater than
15 about 45,000, 55,000, 60,000, 80,000, 90,000, 95,000 or 100,000 USP Units/mg.

2324. The glycoprotein of claim 2021, wherein the potency is about 60,000 to 80,000 USP Units/mg.

20 2425. The glycoprotein of claims 1 or 2021, wherein the glycoprotein is modified with a polymer.

2526. The glycoprotein of claim 2425, wherein the polymer is PEG or dextran.

2627. A composition comprising the glycoprotein of claim 2021.

2728. A nucleic acid molecule comprising a sequence of nucleotides that encodes a ~~hyaluronidase polypeptide glycoprotein~~ of claim 1 or claim 13-12, wherein
5 the sequence of nucleotides includes a stop codon, whereby upon expression and secretion in a cell a polypeptide of claim 1 or 13-12 is produced.

2829. The nucleic acid molecule of claim 2728 that has a sequence of nucleotides set forth in SEQ ID NO:48 or that has a sequence of nucleotides set forth as
10 nucleotides 106-1446 of SEQ ID NO:6.

2930. A vector having a sequence as set forth in SEQ ID NO:51.

3031. A vector comprising the nucleic acid molecule of claim 2728 or claim
15 2829.

3132. The vector of claim 3031 that is an expression vector.

3233. The vector of claim 3031 that is a eukaryotic vector.
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3334. The vector of claim 3031 that includes a sequence of nucleotides that directs secretion of any polypeptide encoded by a sequence of nucleotides operatively linked thereto.

3435. The vector of claim 3034 that is a Pichia vector, an E. coli vector or a viral vector.

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3536. An isolated cell, comprising the vector of claim 3034.

3637. The cell of claim 3536 that is a prokaryotic cell or a eukaryotic cell.

3738. The cell of claim 3536 selected from a bacterial cell, a yeast cell, a plant cell, an insect cell or an animal cell.

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3839. The cell of claim 3536 that is a mammalian cell.

3940. A method for producing a glycoprotein of claim 1, comprising:

introducing a nucleic acid that encodes the polypeptide of claim 1

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operably linked to a suitable promoter into a cell capable of incorporating N-linked sugar moieties into the polypeptide;

culturing the cell under conditions whereby the encoded polypeptide is expressed by the cell and secreted into the cell culture medium; and

recovering the secreted ~~expressed~~-polypeptide.

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4041. The method of claim 3940, wherein the cell is a eukaryotic cell.

4142. The method of claim 4041, wherein the eukaryotic cell is selected from a mammalian cell, an insect cell, a yeast cell or a plant cell.

4243. The method of claim 4142, wherein the cell is a CHO cell.

4344. A method for producing a ~~hyaluronidase~~-glycoprotein, comprising:

5 culturing the cell of claim 3536 under conditions whereby the encoded polypeptide is expressed by the cell; and
 recovering the expressed polypeptide.

4445. The use of the purified glycoprotein of claim 1 in the manufacture of a
10 medicament.

4546. A composition, comprising a substantially purified glycoprotein of claim 1 and a suitable pharmaceutical carrier.

15 4647. The use of the glycoprotein of claim 1 in the manufacture of a medicament for treating an excess of glycosaminoglycan substrate in a subject, wherein the medicament removes said glycosaminoglycan substrate.

4748. The use of claim 4647, wherein said excess substrate is produced from a
20 scar tissue.

4849. The use of claim 4748, wherein said scar tissue is a glial scar resulting from spinal cord injury.

4950. The use of claim 4748, wherein said scar tissue is a result of surgery.

5051. The use of claim 4748, wherein said scar tissue is a keloid scar.

5 5152. The use of claim 5051, wherein said substrate is associated with a
herniated disk.

5253. The use of the glycoprotein of claim 1 for the manufacture of a
medicament, for increasing the diffusion of a therapeutic substance smaller than about
10 0.5 microns in diameter in a subject, wherein the medicament opens or forms channels
smaller than about 0.5 microns in diameter in said subject.

5354. The use of claim 5253, wherein said glycoprotein is injected at a dose
between 0.1 and 1500 USP Units.
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5455. The use of claim 5253, wherein said glycoprotein polypeptide is mixed
with the therapeutic substance prior to injection.

5556. A kit, comprising:

- 20 (a) the glycoprotein of claim 1 at a dose between 0.1 and 1500 USP Units/ml in
an acceptable carrier;
 (b) at least one therapeutic substance in an acceptable carrier; and
 (c) optionally, instructions for delivering therapeutic substances.

5657. The kit of claim 5556, wherein the glycoprotein and the therapeutic substance are provided as a mixture.

5758. The use of claim 4445, wherein the medicament removes excess
5 glycosaminoglycans in a cardiovascular disorder.

5859. The use of claim 5758, wherein said cardiovascular disorder is selected from a myocardial infarct, congestive heart failure or arteriosclerosis.

10 5960. The use of claim 4445, wherein the medicament removes excess glycosaminoglycans in a tumor.

6061. The use of the glycoprotein of claim 1 for the manufacture of a medicament for increasing delivery of a molecule to a tissue containing excess amounts
15 of glycosaminoglycan, wherein the medicament degrades glycosaminoglycans sufficiently to open channels less than about 500 nm in diameter.

6162. The use of claim 6061, wherein the polypeptide is modified with a polymer.

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6263. The use of claim 6162, wherein the polymer is PEG or dextran.

6364. A pharmaceutical composition, comprising the substantially purified glycoprotein of claim 1 and a pharmaceutical carrier.

6465. A pharmaceutical composition, comprising the substantially purified glycoprotein of claim 1.

5 6566. The pharmaceutical composition of claim 64, wherein the glycoprotein is formulated in the composition with a pharmaceutically active agent.

6667. The pharmaceutical composition of claim 6566, wherein the pharmaceutically active agent is selected from the group of agents consisting of a

10 chemotherapeutic agent, an analgesic agent, an anti-inflammatory agent, an antimicrobial agent, an amoebicidal agent, a ~~trichomonocidal~~ trichomonocidal agent, an anti-parkinson agent, an anti-malarial agent, an anticonvulsant agent, an anti-depressant agent, ~~and an antiarthritides~~ antiarthritic agent, an anti-fungal agent, an antihypertensive agent, an antipyretic agent, an anti-parasite agent, an antihistamine agent, an ~~alpha-~~

15 ~~adrenergic~~ alpha-adrenergic agonist agent, an alpha blocker agent, an anesthetic agent, a bronchial dilator agent, a biocide agent, a bactericide agent, a bacteriostat agent, a beta-adrenergic blocker agent, a calcium channel blocker agent, a cardiovascular drug agent, a contraceptive agent, a decongestant agent, a diuretic agent, a depressant agent, a diagnostic agent, an electrolyte agent, a hypnotic agent, a hormone agent, a

20 hyperglycemic agent, a muscle relaxant agent, a muscle contractant agent, an ophthalmic agent, a parasympathomimetic agent, a psychic energizer agent, a sedative agent, a sympathomimetic agent, a tranquilizer agent, an urinary agent, a vaginal agent, a viricide agent, a vitamin agent, a non-steroidal anti-inflammatory agent, an angiotensin converting enzyme inhibitor agent, a polypeptide, a protein, a nucleic acid,

a drug, a ~~an~~ organic molecule, a sleep inducer, an insulin, a cytokine, an antibody and a monoclonal antibody.

5 6768. The pharmaceutical composition of claim 6667, wherein the chemotherapeutic agent is a toxin or a tumor necrosis factor.

6869. The pharmaceutical composition of claim 6667, wherein the anesthetic agent is lidocaine or bupivacaine ~~bupivacaine~~.

10 6970. The pharmaceutical composition of claim 6667, further comprising a hormonal agent.

7071. The pharmaceutical composition of claim 6970, wherein the hormonal agent is epinephrine.

15 7172. The pharmaceutical composition of claim 6364, wherein the pharmaceutical carrier comprises a stabilizing solution.

20 7273. The pharmaceutical composition of claim 7172, wherein the stabilizing solution comprises, NaCl and a metal selected from CaCl₂ and MgCl₂.

7374. The pharmaceutical composition of claim 7172, wherein the stabilizing solution further comprises ethylenediamine tetra-acetic acid (EDTA).

7475. The pharmaceutical composition of claim 7172, wherein the stabilizing solution further comprises a carrier selected from the group comprising albumin, detergent or a surfactant.

5 7576. The pharmaceutical composition of claim 7172, wherein the stabilizing solution comprises phenol red, human serum albumin, HEPES, NaCl and a metal selected from CaCl_2 and MgCl_2 .

10 7677. The pharmaceutical composition of claim 7576, wherein the concentration of the glycoprotein is about 80 USP Units/ml.

7778. The pharmaceutical composition of claim 6364, further comprising a surfactant.

15 7879. The pharmaceutical composition of claim 7778, wherein the surfactant is selected from sorbitan monolaurate; sorbitan monooleate; sorbitan palmitate; sorbitan monostearate; sorbitan sesquioleate-sesquitolate; sorbitan trioleate; polyoxyethylene oleic acid ester derivatives; polyoxyethylene lauryl amine derivatives; polyoxyethylene stearyl amine derivatives; polyoxyethylene oleyl amine derivatives; polyoxyethylene
20 castor oil derivatives; polyoxyethylene hydrogenated castor oil derivatives; polyoxyethylene bis phenol ether derivatives; polyoxyethylene glycols; sorbitan fatty acid ester derivatives; polyoxyethylene sorbitan fatty acid ester derivatives; polyoxyethylene-polyoxypropylene derivatives; polyethylene glycol hexadecyl ether (Brij[®] 56); polyethylene glycol octadecyl ether (Brij[®] 72); polyoxyethylene 10 oleyl

ether (Brij[®] 97); t-Octylphenoxypolyethoxyethanol (TRITON[®] X 100); polyethylene glycol sorbitan monolaurate (TWEEN[®] 20); ~~polyoxyethylenesorbitan~~ polyoxyethylene sorbitan ~~monopalmitate~~ (TWEEN[®] 40); polyethylene glycol sorbitan monostearate (TWEEN[®] 60); ~~polyoxyethylenesorbitan~~ polyoxyethylene sorbitan Tristearate (TWEEN[®] 65); polyethylene glycol sorbitan monooleate (TWEEN[®] 80); ~~polyoxyethylenesorbitan~~ polyoxyethylene sorbitan Trioleate (TWEEN[®] 85); tris(hydroxymethyl) aminomethane lauryl sulfate (TRIZMA[®] dodecyl sulfate); block copolymer of polyethylene and polypropylene glycol (Pluronic[®] F68); or albumin.

10 7980. The pharmaceutical composition of claim 6364, wherein the concentration of the glycoprotein is between about 1 Unit/mg and 5000 USP Units/mg.

8084. The pharmaceutical composition of claim 7980, wherein the concentration of the glycoprotein is between about 1 Unit/mg and 300 USP Units/mg.

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8182. The pharmaceutical composition of claim 7980, wherein the concentration of the glycoprotein is between about 1 Unit/mg and 150 USP Units/mg.

8283. The pharmaceutical composition of claim 6364, further comprising a
20 metal ion selected from calcium and magnesium.

8384. The pharmaceutical compositions of claim 6364 that is formulated as a timed release or that is released upon contact with the site of application or targeted tissue.

8485. The pharmaceutical composition of claim 6364 that is formulated for topical, local, enteric, parenteral, intracystal, intracutaneous, intravitreal, subcutaneous, intramuscular, or intravenous administration.

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8586. The pharmaceutical composition of claim 6364 that is formulated as spray, foam or aerosol, tablet or capsule.

8687. The pharmaceutical composition of claim 8583, that is a tablet or capsule
10 and comprises further comprising an enteric coating.

8788. The pharmaceutical composition of claim 8687, wherein the coating is selected from cellulose acetate phthalate, polyethylene glycol, polyoxyethylene sorbitan, castor oil, ethyl cellulose pseudolatex, phenyl salicylate, n-butyl stearate,
15 stearic acid and carnauba wax.

8889. The pharmaceutical composition of claim 6364 that is a lyophilized powder.

20 8990. The pharmaceutical composition of claim 8889, wherein the lyophilized powder is produced by a process, comprising:

- (a) transferring the pharmaceutical composition into a buffer solution;
- (b) sterile-filtering the resulting solution; and

(c) lyophilizing the filtered solution under standard conditions to produce a sterile powder.

5 9094. The pharmaceutical composition of claim 8990, wherein the buffer solution is selected from HEPES, Phosphate, Citrate and Bicarbonate.

9192. The pharmaceutical composition of claim 9094, wherein the buffer solution further comprises a sugar or carbohydrate.

10 9293. The pharmaceutical composition of claim 9192, wherein the sugar or carbohydrate is dextrose or lactose.

9394. The pharmaceutical composition of claim 9293, wherein the lactose is present at a concentration of between about 1 mg/ml to about 15 mg/ml.

15 9495. The pharmaceutical composition of claim 8990, wherein the buffer further comprises a surfactant.

9596. The pharmaceutical composition of claim 9490, wherein the surfactant
20 is selected from sorbitan monolaurate; sorbitan monooleate; sorbitan palmitate; sorbitan monostearate; sorbitan sesquiolate-sesquitolate; sorbitan trioleate; polyoxyethylene oleic acid ester derivatives; polyoxyethylene lauryl amine derivatives; polyoxyethylene stearyl amine derivatives; polyoxyethylene oleyl amine derivatives; polyoxyethylene castor oil derivatives; polyoxyethylene hydrogenated castor oil derivatives;

polyoxyethylene bis phenol ether derivatives; polyoxyethylene glycols; sorbitan fatty acid ester derivatives; polyoxyethylene sorbitan fatty acid ester derivatives; polyoxyethylene-polyoxypropylene derivatives; polyethylene glycol hexadecyl ether (Brij[®] 56); polyethylene glycol octadecyl ether (Brij[®] 72); polyoxyethylene 10 oleyl ether (Brij[®] 97); t-Octylphenoxypolyethoxyethanol (TRITON[®] X 100); polyethylene glycol sorbitan monolaurate (TWEEN[®] 20); ~~polyoxyethylenesorbitan~~ polyoxyethylene sorbitan monopalmitate (TWEEN[®] 40); polyethylene glycol sorbitan monostearate (TWEEN[®] 60); ~~polyoxyethylenesorbitan~~ polyoxyethylene sorbitan Tristearate (TWEEN[®] 65); polyethylene glycol sorbitan monooleate (TWEEN[®] 80); ~~polyoxyethylenesorbitan~~ polyoxyethylene sorbitan Trioleate (TWEEN[®] 85); tris(hydroxymethyl) aminomethane lauryl sulfate (TRIZMA[®] dodecyl sulfate); block copolymer of polyethylene and polypropylene glycol (Pluronic[®] F68); or albumin.

9697. A combination comprising,
 15 a sterile vial and the pharmaceutical composition of claim 6364 wherein the composition is contained in the vial.

9798. The combination of claim 9697, wherein the sterile vial contains an amount of the pharmaceutical composition that is for single dose administration.

20

9899. A combination comprising, a sterile vial containing the pharmaceutical composition of claim 8485.

99100. The combination of claim 9697, wherein the sterile vial further contains an amount of sterile water for injection; and the final concentration of the glycoprotein is between about 1 and 5000 USP Units/ml.

5 100101. A combination comprising, a sterile syringe containing the pharmaceutical composition of claim 6364.

101102. The combination of claim 100101, wherein the sterile syringe has a volume of between about 5 to 50 μ l.

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102103. The combination of claim 100101, wherein the final concentration of the glycoprotein is between about 1 and about 5000 USP Units/ml.

103104. A combination comprising, a sterile syringe containing the
15 pharmaceutical composition of claim 8485.

104105. The combination of claim 103104, wherein the final concentration of the glycoprotein is between about 1 and about 5000 USP Units/ml.

20 105106. The combination of claim 103104, wherein the pharmaceutical composition is dissolved in about 5 μ l to about 50 μ l of sterile water.

106107. The combination of claim 103104, further comprising a second syringe containing a pharmaceutically effective agent.

107108. The combination of claim ~~106104~~, wherein the pharmaceutically
 effective agent is selected from a chemotherapeutic agent, an analgesic agent, an anti-
 inflammatory agent, an antimicrobial agent, an amoebicidal agent, a ~~trichomonocidal~~
 5 trichomonacidal agent, an anti-parkinson agent, an anti-malarial agent, an
 anticonvulsant agent, an anti-depressant agent, and ~~antiarthritics~~ antiarthritic agent, an
 anti-fungal agent, an antihypertensive agent, an antipyretic agent, an anti-parasite agent,
 an antihistamine agent, an ~~alpha-adrenergic~~ alpha-adrenergic agonist agent, an alpha
 blocker agent, an anesthetic agent, a bronchial dilator agent, a biocide agent, a
 10 bactericide agent, a bacteriostat agent, a beta-adrenergic blocker agent, a calcium
 channel blocker agent, a cardiovascular drug agent, a contraceptive agent, a
 decongestant agent, a diuretic agent, a depressant agent, a diagnostic agent, an
 electrolyte agent, a hypnotic agent, a hormone agent, a hyperglycemic agent, a muscle
 relaxant agent, a muscle contractant agent, an ophthalmic agent, a
 15 parasympathomimetic agent, a psychic energizer agent, a sedative agent,
~~asymphathomimetic~~ a sympathomimetic agent, a tranquilizer agent, an urinary agent, a
 vaginal agent, a viricide agent, a vitamin agent, a non-steroidal anti-inflammatory agent,
 an angiotensin converting enzyme inhibitor agent, a polypeptide, a protein, a nucleic
 acid, a drug, ~~a~~ an organic molecule, a sleep inducer, ~~and~~ an insulin, a cytokine, an
 20 antibody and a monoclonal antibody.

108109. The combination of claim ~~106108~~, wherein the pharmaceutically
 effective agent is a viscoelastic.

109110. A kit comprising the combination of ~~claim 77 or claim 103104~~
and one or more of the following:

(a) packaging material; and

(b) instructions for using the kit for the use of the pharmaceutical composition.

5

110111. The kit of claim 109110, wherein the packaging material includes
ice, dry ice, styrofoam, foam, plastic, cellophane, shrink wrap, bubble wrap, paper,
cardboard, starch peanuts, twist ties, metal clips, metal cans, drierite, glass and rubber.

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111112. The use of claim 4445 in the treatment of a pathologic
accumulation of glycosaminoglycans, wherein said glycoprotein is free of bovine, ovine
or bacterial enzymes, and ameliorates or reduces the accumulated glycosaminoglycan.

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112113. The use of claim 111112, wherein the accumulation is selected
from cardiovascular, cerebral, paraphimosis, myxedema, scleromyxedema and
lymphedema.

20

113114. The use of claim 4445 in facilitating revascularization of necrotic
tissue, wherein the glycoprotein induces the growth of neovasculature into said necrotic
tissue.

114115. The use of the glycoprotein of claim 1 for removal of
glycosaminoglycans from the vitreous humor of a mammalian eye.

115446. The glycoprotein of claim 1 wherein said N-linked glycan demonstrates a degree of polymerization of about 15.6, 13.7, 11.6, 10.0, 8.37, 7.32, 6.1, 5.6, 3.8 or 3.2.

5 | 116447. The method of claim 3940, wherein the cells ~~are~~is cultured in the presence of 0.1-1 mM Sodium Butyrate under conditions suitable for production of the polypeptide.

117448. The method of claim 3940, wherein the method of recovering the
10 expressed polypeptide, comprises:

contacting the ~~media-cell~~ culture medium containing the expressed
polypeptide under low ionic strength with an anion exchange resin at neutral pH:

eluting the polypeptide with about 400 mM of a salt;

contacting the polypeptide with hydrophobic interaction
15 chromatography resin in the presence of about 0.5 M ammonium sulfate;

contacting the polypeptide in ammonium sulfate with phenyl boronate
resin;

eluting the polypeptide in low salt at neutral pH;

contacting the polypeptide with ~~Hydroxapatite~~Hydroxyapatite resin;

20 and

eluting the polypeptide with about 100 mM Na Phosphate, thereby
resulting in a polypeptide that is substantially purified.

~~118119.~~ The use of claim ~~4445~~ for the manufacture of a medicament for treating a disorder of a mammalian eye by inducing liquefaction of ~~a~~ the vitreous humor of the mammalian eye.

5 ~~119120.~~ The use of claim ~~118119~~, wherein the polypeptide is modified with a polymer.

~~120121.~~ The use of claim ~~119120~~, wherein the polymer is PEG or dextran.

10 ~~121122.~~ The use of a glycoprotein of claim 1 in the manufacture of a medicament, for reducing intraocular pressure in the eye of a subject, wherein the glycoprotein is substantially free of bovine, ovine or bacterial enzymes; and is greater than about 40,000 USP Units/mg protein.

15 ~~122123.~~ The use of claim ~~121122~~, wherein the glycoprotein is further characterized by being free of IgG.

~~123124.~~ The use of the glycoprotein of claim 1 in the manufacture of a medicament for treating cardiovascular disorder including myocardial infarct,
20 congestive heart failure and arteriosclerosis, glycosaminoglycan accumulation, vitreous hemorrhage, herniated disc, cancer, intraocular pressure and myxedema, wherein the glycoprotein is present in an amount sufficient to remove excess glycosaminoglycans.