culturing the cell under conditions whereby an encoded modified PH20 polypeptide is produced and secreted by the cell; and recovering the expressed polypeptide.

In one aspect, there is provided a method of producing a modified PH20 polypeptide as described herein, comprising:

introducing a vector, comprising a nucleic acid encoding a modified PH20 polypeptide as described herein into a cell capable of incorporating N-linked sugar moieties into the polypeptide;

culturing the cell under conditions whereby the encoded modified PH20 polypeptide is produced and secreted by the cell; and

recovering the modified PH20 polypeptide.

In one aspect there is provided, a pharmaceutical composition, comprising a modified PH20 polypeptide as described herein, and a pharmaceutically acceptable excipient.

Provided are modified PH20 polypeptides that have an altered property or properties compared to the PH20 polypeptide that do not have the modification(s). The modifications include amino acid replacement, deletion and/or insertions. Detailed structure/function of virtually each amino acid in a PH20 polypeptide is provided herein, as well as the identification of residues and loci that contribute to alteration of a property, such as stability in particular conditions, is provided. Hence, provided are modified PH20 polypeptides that contain one or more amino acid replacements that result in a PH20 polypeptide that retains activity and/or exhibits increased or altered stability under a variety of conditions. Activity retained can be, for example, hyaluronidase activity that is as least about 40% or more of the PH20 polypeptide that does not include the replacement. Exemplary modifications are amino acid replacements. For purposes herein, amino acid replacements are denoted by the single amino acid letter followed by the corresponding amino acid position in SEQ ID NO:3 in which the replacement occurs. Single amino acid abbreviations for amino acid residues are well known to a skilled artisan (*see e.g.* Table 1), and are used herein throughout the description and examples. For example, replacement with P at a position corresponding to position 204 in a PH20 polypeptide with reference to amino acid residue positions set forth in SEQ ID NO:3 means that the replacement encompasses F204P in a PH20 polypeptide set forth in SEQ ID NO:3, or the same replacement at the corresponding position in another PH20 polypeptide.

Provided are modified PH20 polypeptides that contain at least one amino acid replacement in a PH20 polypeptide, whereby the modified PH20 polypeptide exhibits increased stability compared to the PH20 polypeptide not containing the amino acid replacement. Increased stability can be manifested as increased resistance to one or more protein conditions that are denaturing to proteins. The stability of modified and unmodified PH20 is compared under the same conditions. Exemplary protein denaturation (or denaturing, used interchangeably herein) conditions include, but are not limited to, elevated temperature greater than 30 °C or about 30 °C, agitation, low salt, including essentially or substantially or no salt, and presence of excipients that tend to denature proteins. Exemplary of such excipients are antiadherent(s), binder(s), coating(s),

17. The modified PH20 polypeptide of claim 15, comprising up to 5 amino acid modifications selected from among amino acid replacements, insertions and deletions.

18. The modified PH20 polypeptide of any of claims 1-15, comprising up to 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, 41, 42, 43, 44, 45, 46, 47, 48,49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90 or more amino acid replacements.

19. The modified PH20 polypeptide of any of claims 1-18 that is modified by one or more of glycosylation, sialation, albumination, farnysylation, carboxylation, hydroxylation, phosphorylation, conjugation to a polymer, conjugation to a multimerization domain, conjugation to a toxin, conjugation to a detectable label or conjugation to a drug

20. The modified PH20 polypeptide of claim 19, wherein the PH20 polypeptide is conjugated to a polymer that is dextran or PEG.

21. A nucleic acid molecule, encoding a modified PH20 polypeptide of any of claims 1-19.

22. A vector, comprising the nucleic acid molecule of claim 21.

23. A cell, comprising the vector of claim 22.

24. A method of producing a modified PH20 polypeptide of any of claims 1-20, comprising:

introducing a vector, comprising a nucleic acid encoding a modified PH20 polypeptide of any of claims 1-20 into a cell capable of incorporating N-linked sugar moieties into the polypeptide;

culturing the cell under conditions whereby the encoded modified PH20 polypeptide is produced and secreted by the cell; and

recovering the modified PH20 polypeptide.

25. A pharmaceutical composition, comprising a modified PH20 polypeptide of any of claims 1-20, and a pharmaceutically acceptable excipient.

26. The pharmaceutical composition of claim 25, comprising at least one phenolic preservative.

27. The pharmaceutical composition of claim 26, wherein the preservative(s) is(are) selected from among phenol, metacresol (m-cresol), benzyl alcohol, and a paraben.

28. The pharmaceutical composition of any of claims 25-27, comprising a therapeutically active agent.

29. The pharmaceutical composition of claim 28, wherein the therapeutic agent is a polypeptide, a protein, a nucleic acid, a drug, a small molecule or an organic molecule.

30. The pharmaceutical composition of claim 28, wherein the therapeutically active agent is selected from among a chemotherapeutic agent, an analgesic agent, an anti-inflammatory agent, an antimicrobial agent, an amoebicidal agent, a trichomonacidal agent, an anti-parkinson agent, an anti-malarial agent, an anticonvulsant agent, an anti-depressant agent, and antiarthritics agent, an anti-fungal agent, an antihypertensive agent, an antipyretic agent, an anti-parasite agent, an antihistamine agent, an 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, a electrolyte agent, a hypnotic agent, a hormone agent, a hyperglycemic agent, a muscle relaxant agent, a muscle contractant agent,