[0006b] In another aspect, the present disclosure provides the use of a composition for the preparation of a medicament for the treatment of Hunter Syndrome wherein the composition comprises purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1, wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and wherein the purified recombinant I2S contains at least 10% bis-phosphorylated oligosaccharides per molecule.

[0006c] In another aspect, the present disclosure provides the use of a composition for the preparation of a medicament for the treatment of Hunter Syndrome wherein the composition comprises purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO: 1, wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and wherein the purified recombinant I2S protein has specific activity of at least 40 U/mg as determined by an *in vitro* sulfate release activity assay using heparin disaccharide as substrate.

[0006d] In another aspect, the present disclosure provides the use of a composition for the preparation of a medicament for the treatment of Hunter Syndrome wherein the composition comprises purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1, wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and wherein the purified recombinant I2S protein has specific activity of at least 20 U/mg as determined by an *in vitro* 4-MUF-SO_4 to 4-MUF conversion assay.

[0006e] In another aspect, the present disclosure provides the use of a composition for the preparation of a medicament for the treatment of Hunter Syndrome wherein the composition comprises purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1, wherein the purified recombinant I2S (i) comprises at least about 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and (ii) contains on average at least 16 sialic acids per molecule.

[0006f] In yet another aspect, the present disclosure provides the use of a composition for the preparation of a medicament for the treatment of Hunter Syndrome wherein the composition comprises purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1, wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-

formylglycine (FGly), and wherein the purified I2S is characterized with a glycan map comprising seven or fewer peak groups selected from the peak groups indicative of neutral (peak group 1), mono-sialylated (peak group 2), di-sialylated (peak group 3), monophosphorylated (peak group 4), tri-sialylated (peak group 5), tetra-sialylated (peak group 6), or diphosphorylated (peak group 7) I2S protein.

[0007] Thus, in one example, the present disclosure provides a method of purifying recombinant I2S protein from an impure preparation using a process based on one or more of anion-exchange chromatography, cation-exchange chromatography, mixed-mode chromatography, and hydrophobic interaction chromatography. In some examples, an inventive method according to the present disclosure involves less than 6 (e.g., less than 5, less than 4, or less than 3) chromatography steps. In some examples, an inventive method

What is claimed is:

1. Use of a composition for the preparation of a medicament for the treatment of Hunter Syndrome wherein the composition comprises purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1, wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), wherein the purified recombinant I2S contains less than 150 ng/mg Host Cell Protein (HCP).

2. The use of claim 1, wherein the purified recombinant I2S comprises at least 75% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly).

3. The use of claim 1, wherein the purified recombinant I2S comprises at least 85% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly).

4. Use of a composition for the preparation of a medicament for the treatment of Hunter Syndrome wherein the composition comprises purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified recombinant I2S contains at least 10% bis-phosphorylated oligosaccharides per molecule.

5. The use of claim 4, wherein the purified recombinant I2S contains at least 50% bis-phosphorylated oligosaccharides per molecule.

6. Use of a composition for the preparation of a medicament for the treatment of Hunter Syndrome wherein the composition comprises purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO: 1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified recombinant I2S protein has specific activity of at least 40 U/mg as determined by an *in vitro* sulfate release activity assay using heparin disaccharide as substrate.

7. The use of claim 6, wherein the purified recombinant I2S protein has specific activity of at least 50 U/mg as determined by an *in vitro* sulfate release activity assay using heparin disaccharide as substrate.

8. The use of claim 6, wherein the purified recombinant I2S protein has specific activity of at least 60 U/mg as determined by an *in vitro* sulfate release activity assay using heparin disaccharide as substrate.

9. Use of a composition for the preparation of a medicament for the treatment of Hunter Syndrome wherein the composition comprises purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified recombinant I2S protein has specific activity of at least 20 U/mg as determined by an *in vitro* 4-MUF-SO_4 to 4-MUF conversion assay.

10. The use of claim 9, wherein the purified recombinant I2S protein has a specific activity of at least 30 U/mg as determined by an *in vitro* 4-MUF-SO_4 to 4-MUF conversion assay.

11. The use of claim 10, wherein the purified recombinant I2S protein has a specific activity of at least 40 U/mg as determined by an *in vitro* 4-MUF-SO_4 to 4-MUF conversion assay.

12. The use of claim 10, wherein the purified recombinant I2S protein has a specific activity of at least 50 U/mg as determined by an *in vitro* 4-MUF-SO_4 to 4-MUF conversion assay.

13. The use of claim 10, wherein the purified recombinant I2S protein has a specific activity of at least 60 U/mg as determined by an *in vitro* 4-MUF-SO_4 to 4-MUF conversion assay.

14. Use of a composition for the preparation of a medicament for the treatment of Hunter Syndrome wherein the composition comprises purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1, wherein the purified recombinant I2S

(i) comprises at least about 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

(ii) contains on average at least 16 sialic acids per molecule.

15. Use of a composition for the preparation of a medicament for the treatment of Hunter Syndrome wherein the composition comprises purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified I2S is characterized with a glycan map comprising seven or fewer peak groups selected from the peak groups indicative of neutral (peak group 1), mono-sialylated (peak group 2), di-sialylated (peak group 3), monophosphorylated (peak group 4), tri-sialylated (peak group 5), tetra-sialylated (peak group 6), or diphosphorylated (peak group 7) I2S protein.

16. The use of claim 15, wherein the purified recombinant I2S protein contains less than 100 ng/mg HCP.

17. The use of claim 15, wherein the purified recombinant I2S protein contains less than 80 ng/mg HCP.

18. The use of any one of claims 1-17, wherein the treatment comprises that the medicament is to be intrathecally administered.