embodiments, the compositions of the invention comprise at least one mRNA molecule having a coding region and optionally, one or more non-coding regions, wherein the mRNA comprises at least one nucleotide residue that incorporates a 4'-thio-substituted furanose ring, and is complexed with a polymer based carrier or a lipid nanoparticle.

[0013] The invention further provides methods of producing a therapeutic protein *in vivo*, comprising administering to a subject at least one mRNA molecule having a coding region and optionally, one or more non-coding regions, wherein the mRNA comprises at least one nucleotide residue that incorporates a 4'-thio-substituted furanose ring, or a composition comprising such mRNA and a carrier. The invention also provides methods of treating a subject in need of a therapeutic protein, comprising administering at least one mRNA molecule having a coding region and optionally, a non-coding region, wherein the mRNA comprises at least one nucleotide residue that incorporates a 4'-thio-substituted furanose ring, or a composition comprising such mRNA and a carrier. In some embodiments, an administered mRNA in a provided method is at least 60 residues in length. Various modified mRNAs described herein may be used for production of therapeutic proteins or for treatment of various diseases, disorders or conditions.

[0013a] The invention further provides the use of the mRNA molecule as disclosed herein or the composition as disclosed herein in the manufacture of a medicament for producing a protein in vivo.

[0014] In some embodiments, the present invention provides a method for producing a protein using a modified mRNA described herein. Such a method of protein production may be used in an *in vitro* cell free system, *in vitro* cell based system, or *in vivo* system. In various embodiments, a suitable mRNA comprises at least one nucleotide residue that incorporates a 4'-thio-substituted furanose ring. In some embodiments, a suitable mRNA comprises up to about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of nucleotide residues (e.g., ATP, CTP, GTP, UTP, and/or non-standard NTPs) that incorporate a 4'-thio-substituted furanose ring. In some embodiments, a provided mRNA comprises a poly(A) or poly(U) tail. In some embodiments, a provided mRNA is at least 60 residues in length.

[0015] In some embodiments, the present invention provides use of a provided mRNA molecule for the manufacture of a medicament that is capable of producing a therapeutic protein *in vivo*.

WHAT IS CLAIMED IS:

1. An mRNA molecule having a coding region and optionally, one or more non-coding regions, wherein the mRNA comprises at least one nucleotide residue that incorporates a 4'-thio-substituted furanose ring.
2. The mRNA molecule of claim 1, wherein at least 1% of the adenosine nucleotide residues incorporate a 4'-thio-substituted furanose ring.
3. The mRNA molecule of claim 1 or claim 2, wherein at least 1% of the guanosine residues incorporate a 4'-thio-substituted furanose ring.
4. The mRNA molecule of any one of claims 1-3, wherein at least 1% of the uridine residues incorporate a 4'-thio-substituted furanose ring.
5. The mRNA molecule of any one of claims 1-4, wherein at least 1% of the cytidine residues incorporate a 4'-thio-substituted furanose ring.
6. The mRNA molecule of any one of claims 1-5, wherein less than 10% of the nucleotide residues incorporate a 4'-thio-substituted furanose ring.
7. The mRNA molecule of any one of claims 1-5, wherein at least 50% of the residues incorporate a 4'-thio-substituted furanose ring.
8. The mRNA molecule of any one of claims 1-5 or 7, wherein at least 99% of the nucleotide residues incorporate a 4'-thio-substituted furanose ring.
9. The mRNA molecule of any one of claims 1-8, wherein the non-coding region comprises a poly-A tail, and wherein the poly-A tail comprises 4'-thio-adenosine residues.

10. The mRNA molecule of claim 9, wherein the poly-A tail is at least about 90 nucleotide residues in length.

11. The mRNA molecule of claim 9 or claim 10, wherein the poly-A tail is at least about 500 nucleotide residues in length.

12. The mRNA molecule of any one of claims 1-11, wherein the mRNA further comprises at least one nonstandard nucleotide residue.

13. The mRNA molecule of claim 12, wherein the nonstandard nucleotide residue is chosen from one or more of 5-methyl-cytidine, pseudouridine, and 2-thio-uridine.

14. The mRNA molecule according to claim 13, wherein the one or more non-standard nucleoside residue is further modified to include a 4'-thio-furanose.

15. The mRNA molecule of any one of claims 1-14, wherein the molecule comprises at least 200 nucleotide residues.

16. The mRNA molecule of any one of claims 1-15, wherein the molecule comprises at least 5000 nucleotide residues.

17. The mRNA molecule of any one of claims 1-16, wherein the coding region encodes a therapeutic protein.

18. The mRNA molecule of claim 17, wherein the therapeutic protein is chosen from erythropoietin, human growth hormone, cystic fibrosis transmembrane conductance regulator (CFTR), insulin, alpha-galactosidase A, alpha-L-iduronidase, iduronate-2-sulfatase, N-acetylglucosamine-1-phosphate transferase, N-acetylglucosaminidase, alpha-glucosaminide acetyltransferase, N-acetylglucosamine 6-sulfatase, N-acetylgalactosamine-4-sulfatase, beta-glucosidase, galactose-6-sulfate sulfatase, beta-galactosidase, beta-glucuronidase,

glucocerebrosidase, heparan sulfamidase, hyaluronidase, galactocerebrosidase, ornithine transcarbamylase (OTC), carbamoyl-phosphate synthetase 1 (CPS1), argininosuccinate synthetase (ASS1), argininosuccinate lyase (ASL), and arginase 1 (ARG1), glucose-6-phosphatase, glucose-6-phosphate translocase, glycogen debranching enzyme, lysosomal alpha-glucosidase, 1,4-alpha-glucan branching enzyme, glycogen phosphorylase, phosphofructokinase, liver phosphorylase, GLUT-2, UDP glycogen synthase, alpha-L-iduronidase, iduronate sulfate silfatase, heparan sulfate sulfamidase, alpha-N-acetylglucose amidase, alpha-glucosaminid-N-acetyltransferase, N-acetylglucosamine-6-sulfate sulfatase, apolipoprotein E, low density lipoprotein receptor (LDLR), Factor VIII, Factor IX, spinal motor neuron 1 (SMN1), phenylalanine hydroxylase, propionyl-CoA carboxylase, porphobilinogen deaminase, pethylmalonyl-CoA mutase, urate oxidase, C1 esterase inhibitor, and acid alpha-glucosidase.

19. A composition comprising at least one mRNA molecule of any one of claims 1-18 and a carrier.

20. The composition of claim 19, wherein the carrier comprises a lipid.

21. The composition of claim 20, wherein the mRNA is encapsulated within a lipid nanoparticle.

22. The composition of any one of claims 19-21, wherein the carrier is a lipid nanoparticle that comprises one or more cationic lipids selected from XTC (2,2-Dilinoley1-4-dimethylaminoethy1-[1,3]-dioxolane), MC3 (((6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate), ALNY-100 ((3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-

3aH-cyclopenta[d] [1 ,3]dioxol-5-amine)), NC98-5 (4,7,13-tris(3-oxo-3-(undecylamino)propyl)-N1,N16-diundecyl-4,7,10,13-tetraazahexadecane-1,16-diamide), DODAP (1,2-dioleyl-3-dimethylammonium propane), HGT4003, ICE, HGT5000, cis or trans HGT5001, DOTAP (1,2-dioleyl-3-trimethylammonium propane), DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium propane), DLinDMA, DLin-KC2-DMA, and C12-200.

23. The composition of claim 21 or claim 22, wherein the lipid nanoparticle comprises one or more helper lipids selected from DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DOPE (1,2-dioleyl-sn-glycero-3-phosphoethanolamine), DPPE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine), DMPE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine), DOPG (,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)), and cholesterol.

24. The composition any one of claims 21-23, wherein the lipid nanoparticle comprises a pegylated lipid.

25. The composition of claim 21, wherein the lipid nanoparticle comprises one or more cationic lipids, one or more helper lipids, and a pegylated lipid

26. The composition of claim 19, wherein the carrier comprises a polymer.

27. The composition of claim 26, wherein the polymer is polyethyleneimine.

28. A method of producing a protein in vivo, comprising administering the mRNA of any one of claims 1-18 or the composition of any one of claims 19-27 to a subject.

29. Use of an mRNA molecule according to any of claims 1-18 for the manufacture of a medicament that is capable of inducing expression of a therapeutic protein *in vivo*.

Claims

1. An mRNA molecule having a coding region and optionally, one or more non-coding regions, wherein the mRNA comprises at least one nucleotide residue that incorporates a 4'-thio-substituted furanose ring.

2. The mRNA molecule of claim 1, wherein at least 1% of the adenosine nucleotide residues incorporate a 4'-thio-substituted furanose ring.

3. The mRNA molecule of claim 1 or claim 2, wherein at least 1% of the guanosine residues incorporate a 4'-thio-substituted furanose ring.

4. The mRNA molecule of any one of claims 1-3, wherein at least 1% of the uridine residues incorporate a 4'-thio-substituted furanose ring.

5. The mRNA molecule of any one of claims 1-4, wherein at least 1% of the cytidine residues incorporate a 4'-thio-substituted furanose ring.

6. The mRNA molecule of any one of claims 1-5, wherein less than 10% of the nucleotide residues incorporate a 4'-thio-substituted furanose ring.

7. The mRNA molecule of any one of claims 1-5, wherein at least 10% of the nucleotide residues incorporate a 4'-thio-substituted furanose ring.

8. The mRNA molecule of any one of claims 1-5, wherein at least 20% of the nucleotide residues incorporate a 4'-thio-substituted furanose ring.

9. The mRNA molecule of any one of claims 1-5, wherein at least 25% of the nucleotide residues incorporate a 4'-thio-substituted furanose ring.

10. The mRNA molecule of any one of claims 1-5, wherein at least 30% of the nucleotide residues incorporate a 4'-thio-substituted furanose ring.

11. The mRNA molecule of any one of claims 1-5, wherein at least 40% of the nucleotide residues incorporate a 4'-thio-substituted furanose ring.

12. The mRNA molecule of any one of claims 1-5, wherein at least 50% of the nucleotide residues incorporate a 4'-thio-substituted furanose ring.

13. The mRNA molecule of any one of claims 1-5 or 7-12, wherein at least 99% of the nucleotide residues incorporate a 4'-thio-substituted furanose ring.

14. The mRNA molecule of any one of claims 1-13, wherein the non-coding region comprises a poly-A tail, and wherein the poly-A tail comprises 4'-thio-adenosine residues.

15. The mRNA molecule of claim 14, wherein the poly-A tail is at least about 90 nucleotide residues in length.

16. The mRNA molecule of claim 14 or claim 15, wherein the poly-A tail is at least about 500 nucleotide residues in length.

17. The mRNA molecule of any one of claims 1-16, wherein the mRNA further comprises at least one nonstandard nucleotide residue.

18. The mRNA molecule of claim 17, wherein the nonstandard nucleotide residue is chosen from one or more of 5-methyl-cytidine, pseudouridine, and 2-thio-uridine.

19. The mRNA molecule according to claim 18, wherein the one or more non-standard nucleoside residue is further modified to include a 4'-thio-furanose.

20. The mRNA molecule of any one of claims 1-19, wherein the molecule comprises at least 200 nucleotide residues.

21. The mRNA molecule of any one of claims 1-20, wherein the molecule comprises at least 5000 nucleotide residues.

22. The mRNA molecule of any one of claims 1-21, wherein the coding region encodes a therapeutic protein.

23. The mRNA molecule of claim 22, wherein the therapeutic protein is chosen from erythropoietin, human growth hormone, cystic fibrosis transmembrane conductance regulator (CFTR), insulin, alpha-galactosidase A, alpha-L-iduronidase, iduronate-2-sulfatase, N-acetylglucosamine-1-phosphate transferase, N-acetylglucosaminidase, alpha-glucosaminide acetyltransferase, N-acetylglucosamine 6-sulfatase, N-acetylgalactosamine-4-sulfatase, beta-glucosidase, galactose-6-sulfate sulfatase, beta-galactosidase, beta-glucuronidase, glucocerebrosidase, heparan sulfamidase, hyaluronidase, galactocerebrosidase, ornithine transcarbamylase (OTC), carbamoyl-phosphate synthetase 1 (CPS1), argininosuccinate synthetase (ASS1), argininosuccinate lyase (ASL), and arginase 1 (ARG1), glucose-6-phosphatase, glucose-6-phosphate translocase, glycogen debranching enzyme, lysosomal alpha-glucosidase, 1,4-alpha-glucan branching enzyme, glycogen phosphorylase, phosphofructokinase, liver phosphorylase, GLUT-2, UDP glycogen synthase, alpha-L-iduronidase, iduronate sulfate silfatase, heparan sulfate sulfamidase, alpha-N-acetylglucose amidase, alpha-glucosaminid-N-acetyltransferase, N-acetylglucosamine-6-sulfate sulfatase, apolipoprotein E, low density lipoprotein receptor (LDLR), Factor VIII, Factor IX, spinal motor neuron 1 (SMN1), phenylalanine hydroxylase, propionyl-CoA carboxylase, porphobilinogen deaminase, pethylmalonyl-CoA mutase, urate oxidase, C1 esterase inhibitor, and acid alpha-glucosidase.

24. A composition comprising at least one mRNA molecule of any one of claims 1-23 and a carrier.

25. The composition of claim 24, wherein the carrier comprises a lipid.

26. The composition of claim 25, wherein the mRNA is encapsulated within a lipid nanoparticle.

27. The composition of any one of claims 24-26, wherein the carrier is a lipid nanoparticle that comprises one or more cationic lipids selected from XTC (2,2-Dilinoley1-4-dimethylaminoethyl-[1,3]-dioxolane), MC3 (((6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate), ALNY-100 ((3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d] [1 ,3]dioxol-5-amine)), NC98-5 (4,7,13-tris(3-oxo-3-(undecylamino)propyl)-N1,N16-diundecyl-4,7,10,13-tetraazahexadecane-1,16-diamide), DODAP (1,2-dioleyl-3-dimethylammonium propane), HGT4003, ICE, HGT5000, cis or trans HGT5001, DOTAP (1,2-dioleyl-3-trimethylammonium propane), DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium propane), DLinDMA, DLin-KC2-DMA, and C12-200.

28. The composition of claim 26 or claim 27, wherein the lipid nanoparticle comprises one or more helper lipids selected from DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DOPE (1,2-dioleyl-sn-glycero-3-phosphoethanolamine), DPPE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine), DMPE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine), DOPG (,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)), and cholesterol.

29. The composition any one of claims 26-28, wherein the lipid nanoparticle comprises a pegylated lipid.

30. The composition of claim 26, wherein the lipid nanoparticle comprises one or more cationic lipids, one or more helper lipids, and a pegylated lipid.

31. The composition of claim 24, wherein the carrier comprises a polymer.

32. The composition of claim 31, wherein the polymer is polyethyleneimine.

33. The use of the mRNA molecule according to any one of claims 1-23 or the composition any one of claims 24-32 in the manufacture of a medicament for producing a protein in vivo.