CLAIMS

1. An engineered polypeptide comprising a nucleic acid binding domain and a nucleic acid release domain,

wherein one or more amino acids of the engineered polypeptide is a modified amino acid, optionally wherein the modification comprises at least one of:

(i) phosphorylation;
(ii) sulfation;
(iii) glycosylation;
(iv) prenylation;
(v) methylation;
(vi) sialylation;
(vii) lipidation and/or lipoylation;
(viii) acetylation;
(ix) hydroxylation;
(x) palmitoylation;
(xi) mannosylation;
(xii) myristoylation;
(xiii) fucosylation;
(xiv) pegylation; and/or
(xv) any combination thereof.

2. The engineered polypeptide of claim 1, wherein the engineered polypeptide comprises a targeting domain.

3. An engineered polypeptide comprising a nucleic acid binding domain and a targeting domain,

wherein one or more amino acids of the engineered polypeptide is a modified amino acid, optionally wherein the modification comprises at least one of:

(i) phosphorylation;
(ii) sulfation;

(iii) glycosylation;
(iv) prenylation;
(v) methylation;
(vi) sialylation;
(vii) lipidation and/or lipoylation;
(viii) acetylation;
(ix) hydroxylation;
(x) palmitoylation;
(xi) mannosylation;
(xii) myristoylation;
(xiii) fucosylation;
(xiv) pegylation; and/or
(xv) any combination thereof;

wherein engineered polypeptide optionally further comprises a nucleic acid release domain.

4. The engineered polypeptide of any one of claims 1-3, wherein each of two or more amino acids of the engineered polypeptide is a modified amino acid.

5. The engineered polypeptide of any one of claims 1-4, wherein at least one of the modified amino acids comprises a modification chain comprising two or more modifications selected from:

(i) phosphorylation;
(ii) sulfation;
(iii) glycosylation;
(iv) prenylation;
(v) methylation;
(vi) sialylation;
(vii) lipidation and/or lipoylation;
(viii) acetylation;
(ix) hydroxylation;

(x) palmitoylation;
(xi) mannosylation;
(xii) myristoylation;
(xiii) fucosylation;
(xiv) pegylation; and
(xv) any combination thereof.

6. The engineered polypeptide of any one of claims 1-5, wherein the modification increases the stability, half-life, and/or bioavailability of the engineered polypeptide.

7. The engineered polypeptide of any one of claims 1-6, wherein the modification increases the affinity and/or avidity of the engineered polypeptide with a binding partner, optionally wherein the binding partner is a receptor, cell, or cell membrane.

8. The engineered polypeptide of any one of claims 1-7, wherein the modification increases the affinity or avidity of the engineered polypeptide with a nucleic acid.

9. The engineered polypeptide of any one of claims 1-8, wherein the modification decreases precipitation and/or aggregation of the engineered polypeptide.

10. The engineered polypeptide of any one of claims 1-9, wherein the nucleic acid binding domain is derived from a histone polypeptide sequence.

11. The engineered polypeptide of any one of claims 1-10, wherein the nucleic acid binding domain is or comprises the amino acid sequence KRHRK.

12. The engineered polypeptide of any one of claims 1-11, wherein the nucleic acid binding domain is or comprises an amino acid sequence that comprises KRHRK, RRRRR, RRLARR, KKAKAAAKPKK, KKDGKKRKR, KKKLK, KKRIRK, RKKSK, KKPKK, or a combination thereof.

13. The engineered polypeptide of any one of claims 1-12, wherein the nucleic acid binding domain is a modified nucleic acid binding domain in that the nucleic acid binding domain comprises one or more modified amino acids.

14. The engineered polypeptide of any one of claims 2-13, wherein the targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 397-422, wherein the targeting domain is phosphorylated.

15. The engineered polypeptide of any one of claims 2-13, wherein the targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 423-428, wherein the targeting domain is sulfated.

16. The engineered polypeptide of any one of claims 2-13, wherein the targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 429-434, wherein the targeting domain is glycosylated.

17. The engineered polypeptide of any one of claims 2-13, wherein the targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 435-440, wherein the targeting domain is prenylated.

18. The engineered polypeptide of any one of claims 2-13, wherein the targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 441-446, wherein the targeting domain is methylated.

19. The engineered polypeptide of any one of claims 2-13, wherein the targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 447-459, wherein the targeting domain is sialylated.

20. The engineered polypeptide of any one of claims 2-13, wherein the targeting domain is a cell attachment targeting domain, a beta galactose binding domain, a fucose binding domain, a heparin binding domain, a sialic acid binding domain, a glycoprotein binding domain, a carbohydrate binding domain, a lysophosphatidic acid binding domain, a cAMP binding domain, a hyaluronan binding domain, a chondroitin sulfate binding domain, an integrin binding domain, a nucleolin binding domain, a collagen binding domain, a clathrin binding domain, a Fc receptor binding domain, an actin binding domain, an endocytosis motif, a nuclear localization signal, or a combination thereof.

21. The engineered polypeptide of any one of claims 2-13, wherein the targeting domain is a cell attachment targeting domain.

22. The engineered polypeptide of claim 21, wherein the cell attachment targeting domain is or comprises an amino acid sequence that comprises WGREERQ, NTQIH, WNNKTPH, TPH, VNRWS, XBBBXXBX, ARKKAAKA, QRR, SRR, WEPSRPFPVD, HRRTRKAPKRIRLPHIR, KRTGQYKLGSKTGPGQK, KKTK, KLRSQLVKK, RRRCGQKKK, BX(7)B, RIQNLLKITNLRIKFVK, KKEKDIMKKTI, KGE, RGD, RGDS, TTVVNPKYEGK, ERMSQIKRLLS, WRHRARS, GFOGER, LFDLM, WGREERQ, QSTEKRG, LPNTG, or a combination thereof.

23. The engineered polypeptide of any one of claims 2-13, wherein the targeting domain is an internalization domain.

24. The engineered polypeptide of claim 23, wherein the internalization domain is or comprises an amino acid sequence that comprises FXDXF, PPSY, FEDNFVP, YIRV, YADW, YTQV, KKRPKP, SSDDE, RRASS, (YXXL)2, LPLTG, LAFTG, or a combination thereof.

25. The engineered polypeptide of any one of claims 2-13, wherein the targeting domain is a cell-type specific targeting domain.

26. The engineered polypeptide of claims 25, wherein the cell-type specific targeting domain is or comprises an amino acid sequence that comprises ASSLNIA, KKEEEKKEEEKKEEE, LIFHKEQ, KFNKPFVFLI, QPEHSST, EYHHYNK, NGR, GEKGEP, KTKKK, KALKKK, KGKKK, CSVTCG, LRE, YKYNLNGRES, YRSL, $KGGK_7$, KKKQYTSIHHG, KDEL, LADQDYTKTA, or a combination thereof.

27. The engineered polypeptide of any one of claims 2-26, wherein the targeting domain is a modified targeting domain in that the targeting domain comprises one or more modified amino acids.

28. The engineered polypeptide of any one of claims 1-27, wherein the nucleic acid release domain is or comprises an amino acid sequence that comprises GRKKRRQRRRPQ, KRH, KSVKKRSVSEIQ, NRRKKRAL, KFERQ, VRGP, NKDS, NRDN, ANNR, or a combination thereof.

29. The engineered polypeptide of any one of claims 1-28, wherein the nucleic acid release domain is a modified nucleic acid release domain in that the nucleic acid release domain comprises one or more modified amino acids.

30. The engineered polypeptide of any one of claims 1-29, further comprising a poly-arginine domain.

31. The engineered polypeptide of claim 30, wherein the poly-arginine domain is a modified poly-arginine domain in that the poly-arginine domain comprises one or more modified amino acids.

32. The engineered polypeptide of any one of claims 1-31, further comprising a nuclear internalization signal or a nuclear import machinery binding domain.

33. The engineered polypeptide of claim 32, wherein the nuclear internalization signal or nuclear import machinery binding domain is or comprises an amino acid sequence that comprises KKKYKLK, KKRKLE, TRSK, HRKRKR, NKRKRK, AEKSKKK, RKSK, KRVK, KRK, LQQTPLHLAVI, RRPR, PRPR, RPPP, RKKRKGK, PAAKRVKLD, KLKIKRPVK, PKKKRKV, QRKRQK, DSPE, FQVT, QSTEKRG, RQGLID, Cyclic RKKH, or a combination thereof.

34. The engineered polypeptide of claim 32 or claim 33, wherein the nuclear internalization signal or a nuclear import machinery binding domain is a modified nuclear internalization signal or a nuclear import machinery binding domain in that the nuclear internalization signal or a nuclear import machinery binding domain comprises one or more modified amino acids.

35. The engineered polypeptide of any one of claims 1-34, further comprising a stability domain.

36. The engineered polypeptide of claim 35, wherein the stability domain is or comprises an amino acid sequence that comprises YTRF, GDAY, LLEE, RKKRRQRRR, YKSL, YENF, FQDL, YIGSR, IKVAV, or a combination thereof.

37. The engineered polypeptide of claim 35 or 36, wherein the stability domain is a modified stability domain in that the stability domain comprises one or more modified amino acids.

38. The engineered polypeptide of any one of claims 1-37, further comprising an oligomerization domain.

39. The engineered polypeptide of claim 38, wherein the oligomerization domain is selected from the oligomerization domains of Table 11, optionally wherein the oligomerization domain is positioned at the C-terminus of the engineered polypeptide.

40. The engineered polypeptide of claim 38 or claim 39, wherein the oligomerization domain is a modified oligomerization domain in that the oligomerization domain comprises one or more modified amino acids.

41. The engineered polypeptide of any one of claims 1-40, wherein the polypeptide comprises a linker.

42. The engineered polypeptide of claim 41, wherein the linker is a linker according to any one of SEQ ID NOs: 154-250.

43. The engineered polypeptide of claim 41 or 42, wherein the linker is a modified linker in that the linker comprises one or more modified amino acids.

44. The engineered polypeptide of any one of claims 1-43, wherein one or more amino acids of the engineered polypeptide is a phosphorylated amino acid.

45. The engineered polypeptide of claim 44, wherein the phosphorylated amino acid is a serine, threonine, or tyrosine amino acid.

46. The engineered polypeptide of claim 44 or claim 45, wherein the phosphorylated amino acid is present in a linker domain or targeting domain.

47. The engineered polypeptide of any one of claims 1-43, wherein one or more amino acids of the engineered polypeptide is a sulfated amino acid.

48. The engineered polypeptide of claim 47, wherein the sulfated amino acid is a serine, threonine, or tyrosine amino acid.

49. The engineered polypeptide of claim 47 or claim 48, wherein the sulfated amino acid is present in a linker domain or targeting domain.

50. The engineered polypeptide of any one of claims 1-43, wherein one or more amino acids of the engineered polypeptide is an acetylated amino acid.

51. The engineered polypeptide of claim 50, wherein the acetylated amino acid is a lysine amino acid.

52. The engineered polypeptide of claim 50 or claim 51, wherein the acetylated amino acid is present in a linker domain or targeting domain.

53. The engineered polypeptide of any one of claims 1-43, wherein one or more amino acids of the engineered polypeptide is a mannosylated amino acid.

54. The engineered polypeptide of claim 53, wherein the mannosylated amino acid is a serine amino acid.

55. The engineered polypeptide of claim 53 or 54, wherein the mannosylated amino acid is present in a linker domain or targeting domain.

56. A polynucleotide that encodes the amino acid sequence of an engineered polypeptide of any one of claims 1-55.

57. The polynucleotide of claim 56, wherein the polynucleotide is DNA or RNA.

58. A vector comprising the polynucleotide of claim 56 or 57.

59. A cell comprising an engineered polypeptide of any one of claims 1-55, a polynucleotide of claim 56 or 57, or a vector of claim 58.

60. A method of making an engineered polypeptide of any one of claims 1-55, the method comprising expressing a polynucleotide of any one of claims 56-58 in a cell.

61. The method of claim 60, further comprising isolating the engineered polypeptide from the cell.

62. A composition comprising:
(i) at least one polynucleotide, and
(ii) at least one engineered polypeptide of any one of claims 1-55.

63. The composition of claim 62, wherein the at least one polynucleotide is or comprises DNA or RNA.

64. The composition of claim 62 or 63, wherein the at least one polynucleotide comprises a nucleotide sequence encoding a polypeptide.

65. The composition of any one of claims 62-64, wherein the at least one polynucleotide is or comprises mRNA.

66. The composition of claim 62 or 63, wherein the at least one polynucleotide comprises an inhibitory RNA.

67. The composition of claim 66, wherein the inhibitory RNA is a gRNA, siRNA, miRNA, or shRNA.

68. The composition of any one of claims 62-67, comprising at least two engineered polypeptides of any one of claims 1-55, wherein a first engineered polypeptide of any one of claims 1-55 is able to oligomerize with a second engineered polypeptide of any one of claims 1-55.

69. The composition of any one of claims 62-68, wherein the ratio of polynucleotides to engineered polypeptides of any one of claims 1-55 is between 1:1 and 1:2,000.

70. The composition of claim 69, wherein the ratio of polynucleotides to engineered polypeptides of any one of claims 1-55 is between 1:1 and 1:1,000, between 1:1 and 1:500, between 1:1 and 1:200, between 1:1 and 1: 100, between 1:1 and 1:50, between 1:3 and 1:1,000, between 1:3 and 1:500, between 1:3 and 1:200, between 1:3 and 1: 100, or between 1:3 and 1:50.

71. The composition of any one of claims 62-70, wherein the ratio of polynucleotides to engineered polypeptides of any one of claims 1-55 is between 1:200 and 1:2,000, between 1:200 and 1:1000, or between 1:200 and 1:500.

72. The composition of any one of claims 62-71, comprising a pharmaceutical carrier.

73. A method comprising administering a composition of any one of claims 62-72 to a system, wherein the system is a cell, tissue, or subject.

74. The method of claim 73, wherein, after administration, the modification increases the stability, half-life, and/or bioavailability of the composition in the system.

75. The method of claim 73 or 74, wherein, after administration, the modification increases the affinity or avidity of the composition with a binding partner in the system, optionally wherein the binding partner is a receptor, cell, or cell membrane.

76. The method of any one of claims 73-75, wherein, after administration, the modification decreases precipitation and/or aggregation of the composition in the system.

77. The method of any one of claims 73-76, wherein, after administration, the modification increases the rate at which the composition enters one or more cells in the system.

78. The method of any one of claims 73-77, wherein, after administration, the modification increases delivery of the composition to one or more cells in the system.

79. The method of any one of claims 73-78, wherein, after administration, the modification increases delivery of the nucleic acid of the composition to one or more cells in the system.

80. The method of any one of claims 73-79, wherein the system is a mammalian subject and, after administration, the modification decreases accumulation of the composition in liver.

81. The method of any one of claims 73-80, wherein the system is a mammalian subject and, after administration, the modification increases the amount of composition that crosses the blood-brain barrier.

82. A method comprising administering a composition of any one of claims 62-72 to a cell, tissue, or subject, wherein one or more amino acids of the engineered polypeptide is a phosphorylated amino acid.

83. The method of claim 82, wherein the phosphorylated amino acid is a serine, threonine, or tyrosine amino acid.

84. The method of claim 82 or claim 83, wherein the phosphorylated amino acid is present in a linker domain or targeting domain.

85. The method of any one of claims 82-84, wherein the composition is delivered to cells of the central nervous system (CNS).

86. The method of any one of claims 82-84, wherein the composition is delivered to CNS neurons.

87. The method of any one of claims 82-84, wherein the composition is delivered to CNS astrocytes, microglia, oligodendrocytes, or glia.

88. The method of any one of claims 82-84, wherein the composition is delivered to spinal cord cells, optionally wherein the spina cord cells are spinal cord neurons or spinal cord glial cells.

89. The method of any one of claims 82-88, wherein the polynucleotide encodes an expression product that is expressed in cells to which the composition is delivered.

90. The method of any one of claims 82-89, wherein the subject is a mammalian subject and the administration is intrathecal, intracranial, or intra-cisterna magna.

91. A method comprising administering a composition of any one of claims 62-72 to a cell, tissue, or subject, wherein one or more amino acids of the engineered polypeptide is a sulfated amino acid.

92. The method of claim 91, wherein the sulfated amino acid is a serine, threonine, or tyrosine amino acid.

93. The method of claim 91 or claim 92, wherein the sulfated amino acid is present in a linker domain or targeting domain.

94. The method of any one of claims 91-93, wherein the composition is delivered to cells of the central nervous system (CNS).

95. The method of any one of claims 91-93, wherein the composition is delivered to CNS neurons.

96. The method of any one of claims 91-93, wherein the composition is delivered to CNS astrocytes, microglia, oligodendrocytes, or glia.

97. The method of any one of claims 91-93, wherein the composition is delivered to spinal cord cells, optionally wherein the spina cord cells are spinal cord neurons or spinal cord glial cells.

98. The method of any one of claims 91-97, wherein the polynucleotide encodes an expression product that is expressed in cells to which the composition is delivered.

99. The method of any one of claims 91-98, wherein the subject is a mammalian subject and the administration is intrathecal, intracranial, or intra-cisterna magna.

100. A method comprising administering a composition of any one of claims 62-72 to a cell, tissue, or subject, wherein one or more amino acids of the engineered polypeptide is an acetylated amino acid.

101. The method of claim 100, wherein the acetylated amino acid is a lysine amino acid.

102. The method of claim 100 or claim 101, wherein the acetylated amino acid is present in a linker domain or targeting domain.

103. The method of any one of claims 100-102, wherein the composition is delivered to CNS neurons.

104. The method of any one of claims 100-102, wherein the composition is delivered to retinal cells.

105. The method of any one of claims 100-102, wherein the composition is delivered to retinal neurons, optionally wherein the retinal neurons include one or more of photoreceptors, bipolar cells, retinal ganglion cells, horizontal cells, and amacrine cells.

106. The method of any one of claims 100-102, wherein the composition is delivered to photoreceptor cells, optionally wherein the photoreceptor cells include one or both of rods and cones.

107. The method of any one of claims 100-106, wherein the polynucleotide encodes an expression product that is expressed in cells to which the composition is delivered.

108. The method of any one of claims 100-107, wherein the subject is a mammalian subject and the administration is intravitreal, suprachoroidal, or subretinal.

109. A method comprising administering a composition of any one of claims 62-72 to a cell, tissue, or subject, wherein one or more amino acids of the engineered polypeptide is a mannosylated amino acid.

110. The method of claim 109, wherein the mannosylated amino acid is a serine amino acid.

111. The method of claim 109 or claim 110, wherein the mannosylated amino acid is present in a linker domain or targeting domain.

112. The method of any one of claims 109-111, wherein the composition is delivered to CNS neurons.

113. The method of any one of claims 109-111, wherein the composition is delivered to retinal cells.

114. The method of any one of claims 109-111, wherein the composition is delivered to retinal neurons, optionally wherein the retinal neurons include one or more of photoreceptors, bipolar cells, retinal ganglion cells, horizontal cells, and amacrine cells.

115. The method of any one of claims 109-111, wherein the composition is delivered to photoreceptor cells include one or both of rods and cones.

116. The method of any one of claims 109-115, wherein the polynucleotide encodes an expression product that is expressed in cells to which the composition is delivered.

117. The method of any one of claims 109-116, wherein the subject is a mammalian subject and the administration is intravitreal, suprachoroidal, or subretinal.

118. A method of condensing a polynucleotide, comprising contacting the polynucleotide with a polypeptide of any one of claims 1-55.

119. A method of neutralizing the charge of a polynucleotide, comprising contacting the polynucleotide with a polypeptide of any one of claims 1-55.

120. A composition comprising an engineered polypeptide and at least one nucleic acid,

the engineered polypeptide comprising a nucleic acid binding domain and a nucleic acid release domain,

wherein one or more amino acids of the engineered polypeptide is a modified amino acid, optionally wherein the modification comprises at least one of:

(i) phosphorylation;
(ii) sulfation;
(iii) glycosylation;
(iv) prenylation;
(v) methylation;
(vi) sialylation;
(vii) lipidation and/or lipoylation;
(viii) acetylation;
(ix) hydroxylation;
(x) palmitoylation;

(xi) mannosylation;
(xii) myristoylation;
(xiii) fucosylation;
(xiv) pegylation; and/or
(xv) any combination thereof;

optionally wherein the composition is for use in delivering a nucleic acid to a subject or system.

121. The composition of claim 120, wherein the engineered polypeptide comprises a targeting domain.

122. A composition comprising an engineered polypeptide and at least one nucleic acid,

the engineered polypeptide comprising a nucleic acid binding domain and a targeting domain,

wherein one or more amino acids of the engineered polypeptide is a modified amino acid, optionally wherein the modification comprises at least one of:

(i) phosphorylation;
(ii) sulfation;
(iii) glycosylation;
(iv) prenylation;
(v) methylation;
(vi) sialylation;
(vii) lipidation and/or lipoylation;
(viii) acetylation;
(ix) hydroxylation;
(x) palmitoylation;
(xi) mannosylation;
(xii) myristoylation;
(xiii) fucosylation;
(xiv) pegylation; and/or
(xv) any combination thereof;

optionally wherein the composition is for use in delivering a nucleic acid to a subject or system.

123. The engineered polypeptide of any one of claims 1-55, the polynucleotide of any one of claims 56-57, the vector of claim 58, the cell of claim 59, the method of any one of claims 60-61, the composition of any one of claims 62-72, 120, 121, or 122, or the method of any one of claims 73-119, wherein the engineered polypeptide has at least 80% sequence identity with an amino acid sequence selected from SEQ ID NOs: 336-388.