

CLAIMS

What is claimed is:

1. A method to determine the serotype of an adeno-associated virus (AAV) particle comprising
 - a) denaturing the AAV particle,
 - b) subjecting the denatured AAV particle to liquid chromatography/mass spectrometry (LC/MS), and
 - c) determining the masses of VP1, VP2 and VP3 of the AAV particle;wherein the specific combination of masses of VP1, VP2 and VP3 are indicative of the AAV serotype.
2. The method of claim 1, wherein the calculated masses of VP1, VP2 and VP3 are compared to the theoretical masses of VP1, VP2 and VP3 of one or more AAV serotypes.
3. A method of determining the heterogeneity of an AAV particle comprising
 - a) denaturing the AAV particle,
 - b) subjecting the denatured AAV particle to liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS),
 - c) determining the masses of VP1, VP2 and VP3 of the AAV particle, and
 - d) comparing the masses of step c) with the theoretical masses of VP1, VP2 and VP3 of the AAV serotype;wherein a deviation of one or more of the masses of VP1, VP2 or VP3 are indicative of the AAV capsid heterogeneity.

4. The method of claim 3 wherein the heterogeneity comprises one or more of mixed serotypes, variant capsids, capsid amino acid substitutions, truncated capsids, or modified capsids.
5. The method of any one of claims 1-4, wherein the AAV particle is denatured with acetic acid, guanidine hydrochloride and/or an organic solvent.
6. The method of any one of claims 1-5, wherein the liquid chromatography is reverse phase liquid chromatography, size exclusion chromatography, hydrophilic interaction liquid chromatography, or cation exchange chromatography.
7. The method of any one of claims 1-6, wherein the liquid chromatography is reverse phase liquid chromatography.
8. The method of claim 7, wherein the reverse phase chromatography is a C4 or C8 reverse chromatography.
9. The method of claim 8, wherein the chromatography uses a mobile phase A comprising formic acid in water.
10. The method of claim 9, wherein the mobile phase A comprises about 0.1% formic acid.
11. The method of any one of claims 8-10, wherein the chromatography comprises a mobile phase B comprising formic acid in acetonitrile.
12. The method of claim 11, wherein the mobile phase B comprises about 0.1% formic acid.

13. The method of claim 11 or 12, wherein the proportion of mobile phase B in the chromatography increases over time.
14. The method of claim 13, wherein the proportion of mobile phase B in the chromatography increases in a stepwise manner.
15. The method of claim 14, wherein mobile phase B increases from about 10% to about 20%, from about 20% to about 30%, and from about 30% to about 38%.
16. The method of claim 15, wherein mobile phase B increases from about 10% to about 20% in about 6 minutes, from about 20% to about 30% in about 10 minutes, and from about 30% to about 38% in about 40 minutes.
17. The method of any one of claims 1-16, wherein the liquid chromatography is ultra-performance liquid chromatography (UPLC).
18. The method of any one of claims 1-17, wherein the mass spectrometry comprises a capillary voltage of about 3.5 kV.
19. The method of any one of claims 1-18, wherein the mass spectrometry comprises a sampling cone voltage of about 45 V.
20. The method of any one of claims 1-19 wherein the mass spectrometry comprises assisted calibration.

21. The method of claim 20, wherein sodium iodide is used as a calibrant.
22. The method of any one of claims 1-21, wherein the N-terminus of VP1 and/or VP3 is acetylated.
23. The method of any one of claims 1-22, wherein the AAV particle is a recombinant AAV (rAAV) particle.
24. The method of any one of claims 1-23, wherein the AAV particle comprises an AAV1 capsid, an AAV2 capsid, an AAV3 capsid, an AAV4 capsid, an AAV5 capsid, an AAV6 capsid, an AAV7 capsid, an AAV8 capsid, an AAVrh8 capsid, an AAV9 capsid, an AAV10 capsid, an AAVrh10 capsid, an AAV11 capsid, an AAV12 capsid, an AAV LK03 capsid, an AAV2R471A capsid, an AAV2/2-7m8 capsid, an AAV DJ capsid, an AAV DJ8 capsid, an AAV2 N587A capsid, an AAV2 E548A capsid, an AAV2 N708A capsid, an AAV V708K capsid, a goat AAV capsid, an AAV1/AAV2 chimeric capsid, a bovine AAV capsid, a mouse AAV capsid, rAAV2/HBoV1 (chimeric AAV / human bocavirus virus 1), an AAV2HBKO capsid, an AAVPHP.B capsid or an AAVPHP.eB capsid.
25. The method of claim 23 or 24, wherein the AAV capsid comprises a tyrosine mutation or a heparin binding mutation.
26. The method of any one of claims 1-25, wherein the masses of VP1, VP2, and VP3 are compared to the theoretical masses of one or more of AAV1 capsid, an AAV2 capsid, an AAV3 capsid, an AAV4 capsid, an AAV5 capsid, an AAV6 capsid, an AAV7 capsid, an AAV8 capsid, an AAVrh8 capsid, an AAV9 capsid, an AAV10 capsid, an AAVrh10 capsid, an AAV11 capsid, an AAV12 capsid, an AAV LK03 capsid, an AAV2R471A capsid, an AAV2/2-7m8 capsid, an AAV DJ capsid, an AAV DJ8 capsid, an AAV2 N587A capsid, an AAV2 E548A capsid, an AAV2 N708A capsid, an AAV V708K capsid, a goat AAV capsid, an AAV1/AAV2 chimeric

capsid, a bovine AAV capsid, a mouse AAV capsid rAAV2/HBoV1 (chimeric AAV / human bocavirus virus 1) , an AAV2HBKO capsid, an AAVPHP.B capsid or an AAVPHP.eB capsid.

27. The method of any one of claims 1-26, wherein the viral particle comprises an AAV1 ITR, an AAV2 ITR, an AAV3 ITR, an AAV4 ITR, an AAV5 ITR, an AAV6 ITR, an AAV7 ITR, an AAV8 ITR, an AAVrh8 ITR, an AAV9 ITR, an AAV10 ITR, an AAVrh10 ITR, an AAV11 ITR, or an AAV12 ITR.

28. The method of any one of claims 1-27, wherein the AAV particle comprises an AAV vector encoding a heterologous transgene.

29. A method to determine the serotype of an adeno-associated virus (AAV) particle comprising

- a) denaturing the AAV particle,
 - b) subjecting the denatured AAV particle to reduction and/or alkylation,
 - c) subjecting the denatured AAV particle to digestion to generate fragments of VP1, VP2 and/or VP3 of the AAV particle,
 - d) subjecting the fragments of VP1, VP2 and/or VP3 to liquid chromatography/mass spectrometry-mass spectrometry (LC/MS/MS), and
 - e) determining the masses of fragments of VP1, VP2 and VP3 of the AAV particle;
- wherein the specific combination of masses of fragments of VP1, VP2 and VP3 are indicative of the AAV serotype.

30. The method of claim 29, wherein the calculated masses of the fragments of VP1, VP2 and/or VP3 are compared to the theoretical masses of fragments of VP1, VP2 and/or VP3 of one or more AAV serotypes.

31. A method of determining the heterogeneity of a serotype of an AAV particle comprising
- a) denaturing the AAV particle,
 - b) subjecting the denatured AAV particle to reduction and/or alkylation,
 - c) subjecting the denatured AAV particle to digestion to generate fragments of VP1, VP2 and/or VP3 of the AAV particle,
 - d) subjecting the fragments of VP1, VP2 and/or VP3 to liquid chromatography/mass spectrometry-mass spectrometry (LC/MS/MS),
 - e) determining the masses of fragments of VP1, VP2 and VP3 of the AAV particle, and
 - f) comparing the masses of step e) with the theoretical masses of fragments of VP1, VP2 and VP3 of the AAV serotype;
- wherein a deviation of one or more of the masses of VP1, VP2 or VP3 are indicative of the AAV capsid heterogeneity.
32. The method of claim 31 wherein the heterogeneity comprises one or more of mixed serotypes, variant capsids, capsid amino acid substitutions, truncated capsids, or modified capsids.
33. The method of any one of claims 29-32, wherein the reduction is by subjecting the AAV particle to dithiothreitol, beta-mercaptoethanol, or tris(2-carboxyethyl)phosphine (TCEP).
34. The method of any one of claims 29-33, where in the alkylation is by subjecting the AAV particle to iodoacetic acid, iodoacetamide, or 4-vinylpyridine.
35. The method of any one of claims 29-34, wherein the digestion is an enzymatic digestion or a chemical digestion.

36. The method of claim 35, wherein the enzymatic digestion is an endopeptidase digestion.
37. The method of claim 35 or 36, wherein the enzymatic digestion is a trypsin digestion, a LysC digestion, an Asp-N digestion or a Glu-C digestion.
38. The method of claim 35, wherein the chemical digestion is cyanogen bromide digestion or an acid digestion.
39. The method of any one of claims 29-37, wherein the AAV particle is denatured with acetic acid, guanidine hydrochloride and/or an organic solvent.
40. The method of any one of claims 29-39, wherein the liquid chromatography is reverse phase liquid chromatography, size exclusion chromatography, hydrophilic interaction liquid chromatography, or cation exchange chromatography.
41. The method of any one of claims 29-40, wherein the liquid chromatography is reverse phase liquid chromatography.
42. The method of claim 41, wherein the reverse phase chromatography is a C18 reverse chromatography.
43. The method of claim 42, wherein the chromatography uses a mobile phase A comprising formic acid in water.
44. The method of claim 43, wherein the mobile phase A comprises about 0.1% formic acid.

45. The method of any one of claims 42-44, wherein the chromatography comprises a mobile phase B comprising formic acid in acetonitrile.
46. The method of claim 45, wherein the mobile phase B comprises about 0.1% formic acid.
47. The method of claim 45 or 46, wherein the proportion of mobile phase B in the chromatography increases over time.
48. The method of claim 47, wherein mobile phase B increases from about 2% to about 60%.
49. The method of claim 48, wherein mobile phase B increases from about 2% to about 60% in about 121 minutes.
50. The method of any one of claims 29-49, wherein the liquid chromatography is high-performance liquid chromatography (HPLC).
51. The method of any one of claims 29-50, wherein the mass spectrometry comprises a source voltage of about 2.5 kV.
52. The method of any one of claims 29-51, wherein the mass spectrometry comprises a capillary temperature of about 275 °C.
53. The method of any one of claims 29-52 wherein the S-lens RF level of the mass spectrometry comprises is about 55%.

54. The method of any one of claims 29-53, wherein the N-terminus of VP1 and/or VP3 is acetylated.
55. The method of any one of claims 29-54, wherein the AAV particle is a recombinant AAV (rAAV) particle.
56. The method of any one of claims 29-55, wherein the AAV particle comprises an AAV1 capsid, an AAV2 capsid, an AAV3 capsid, an AAV4 capsid, an AAV5 capsid, an AAV6 capsid, an AAV7 capsid, an AAV8 capsid, an AAVrh8 capsid, an AAV9 capsid, an AAV10 capsid, an AAVrh10 capsid, an AAV11 capsid, an AAV12 capsid, an AAV LK03 capsid, an AAV2R471A capsid, an AAV2/2-7m8 capsid, an AAV DJ capsid, an AAV DJ8 capsid, an AAV2 N587A capsid, an AAV2 E548A capsid, an AAV2 N708A capsid, an AAV V708K capsid, a goat AAV capsid, an AAV1/AAV2 chimeric capsid, a bovine AAV capsid, a mouse AAV capsid rAAV2/HBoV1 (chimeric AAV / human bocavirus virus 1), an AAV2HBKO capsid, an AAVPHP.B capsid or an AAVPHP.eB capsid.
57. The method of claim 55 or 56, wherein the AAV capsid comprises a tyrosine mutation, a heparin binding mutation, or an HBKO mutation.
58. The method of any one of claims 29-57, wherein the masses of VP1, VP2, and VP3 are compared to the theoretical masses of one or more of AAV1 capsid, an AAV2 capsid, an AAV3 capsid, an AAV4 capsid, an AAV5 capsid, an AAV6 capsid, an AAV7 capsid, an AAV8 capsid, an AAVrh8 capsid, an AAV9 capsid, an AAV10 capsid, an AAVrh10 capsid, an AAV11 capsid, an AAV12 capsid, an AAV LK03 capsid, an AAV2R471A capsid, an AAV2/2-7m8 capsid, an AAV DJ capsid, an AAV DJ8 capsid, an AAV2 N587A capsid, an AAV2 E548A capsid, an AAV2 N708A capsid, an AAV V708K capsid, a goat AAV capsid, an AAV1/AAV2 chimeric capsid, a bovine AAV capsid, a mouse AAV capsid rAAV2/HBoV1 (chimeric AAV / human bocavirus virus 1), an AAV2HBKO capsid, an AAVPHP.B capsid or an AAVPHP.eB capsid.

59. The method of any one of claims 29-58, wherein the viral particle comprises an AAV1 ITR, an AAV2 ITR, an AAV3 ITR, an AAV4 ITR, an AAV5 ITR, an AAV6 ITR, an AAV7 ITR, an AAV8 ITR, an AAVrh8 ITR, an AAV9 ITR, an AAV10 ITR, an AAVrh10 ITR, an AAV11 ITR, or an AAV12 ITR.
60. The method of any one of claims 29-59, wherein the AAV particle comprises an AAV vector encoding a heterologous transgene.
61. A method of determining the serotype of an AAV particle comprising the method of any one of claims 1, 2 or 4-28 combined with the method of any one of claims 29, 30 or 32-59.
62. A method of determining heterogeneity of an AAV particle comprising the method of any one of claims 3-28 combined with the method of any one of claims 31-60.
63. A recombinant AAV (rAAV) particle comprising an amino acid substitution at amino acid residue 2 of VP1 and/or VP3 or a parent polypeptide; wherein the amino acid substitution at amino acid residue 2 of VP1 and/or VP3 alters N-terminal acetylation compared to N-terminal acetylation at amino acid residue 2 of VP1 and/or VP3 of the parent AAV particle.
64. The rAAV particle of claim 63, wherein the substitution results in a higher frequency of N-terminal acetylation or a lower frequency of N-terminal acetylation.
65. The rAAV particle of claim 63 or 64, wherein the rAAV particle comprises an amino acid substitution at amino acid residue 2 of VP1; and wherein the amino acid substitution at amino acid residue 2 of VP1 alters N-terminal acetylation compared to N-terminal acetylation at amino acid residue 2 of VP1 of the parent AAV particle.

66. The rAAV particle of any one of claims 63-65, wherein the rAAV particle comprises an amino acid substitution at amino acid residue 2 of VP3; and wherein the amino acid substitution at amino acid residue 2 of VP3 alters N-terminal acetylation compared to N-terminal acetylation at amino acid residue 2 of VP3 of the parent AAV particle.

67. The rAAV particle of any one of claims 63-66, wherein the amino acid residue 2 is substituted with Cys, Ser, Thr, Val, Gly, Asn, Asp, Glu, Ile, Leu, Phe, Gln, Lys, Met, Pro or Tyr.

68. The rAAV particle of claim 67, wherein the amino acid residue 2 is substituted with Ser, Asp or Glu.

69. The rAAV particle of any one of claims 63-68, wherein the AAV particle comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV LK03, AAV2R471A, AAV2/2-7m8, AAV DJ, an AAV DJ8 capsid, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV V708K, goat AAV, AAV1/AAV2 chimeric, bovine AAV, mouse AAV, rAAV2/HBoV1 serotype capsid, AAV2 HBKO, AAVPHP.B or AAVPHB.eB serotype capsid.

70. The rAAV particle of any one of claims 63-69, comprising a tyrosine mutation or a heparin binding mutation in the particle's capsid.

71. The rAAV particle of any one of claims 63-70, wherein the rAAV particle comprises a rAAV vector.

72. The rAAV particle of claim 71, wherein the rAAV vector comprises one or more AAV ITRs.

73. The rAAV particle of claim 71 or 72, wherein the rAAV vector comprises an AAV1 ITR, an AAV2 ITR, an AAV3 ITR, an AAV4 ITR, an AAV5 ITR, an AAV6 ITR, an AAV7 ITR, an AAV8 ITR, an AAVrh8 ITR, an AAV9 ITR, an AAV10 ITR, an AAVrh10 ITR, an AAV11 ITR, or an AAV12 ITR.

74. The rAAV particle of claim 72 or 73, wherein the AAV capsid and the AAV ITRs are derived from the same serotype.

75. The rAAV particle of claim 72 or 73, wherein the AAV capsid and the AAV ITRs are derived from different serotypes.

76. The rAAV particle of any one of claims 71-75, wherein the AAV particle comprises an AAV vector encoding a heterologous transgene flanked by one or more AAV ITRs.

77. The rAAV particle of any one of claims 71-76, wherein the rAAV vector is a self-complementary vector.

78. The rAAV particle of claim 77, wherein the rAAV vector comprises a first nucleic acid sequence encoding the transgene and a second nucleic acid sequence encoding a complement of the transgene, wherein the first nucleic acid sequence can form intrastrand base pairs with the second nucleic acid sequence along most or all of its length.

79. The rAAV particle of claim 78, wherein the first nucleic acid sequence and the second nucleic acid sequence are linked by a mutated AAV ITR, wherein the mutated AAV ITR comprises a deletion of the D region and comprises a mutation of the terminal resolution sequence.

80. The rAAV particle of any one of claims 63-79, wherein the rAAV particle is produced by transfecting a host cell with nucleic acid encoding the rAAV vector and nucleic acid encoding AAV rep and cap functions, and providing nucleic acid encoding AAV helper functions.

81. The rAAV particle of claim 80, wherein the AAV helper functions are provided by transfecting the host cell with nucleic acid encoding the AAV helper functions.

82. The rAAV particle of claim 81, wherein the AAV helper functions are provided by infecting the host cell with an AAV helper virus that provides the AAV helper functions.

83. The rAAV particle of claim 82, wherein the AAV helper virus is an adenovirus, a herpes simplex virus or a baculovirus.

84. The rAAV particle of any one of claims 63-79, wherein the rAAV particle is produced by an AAV producer cell comprising nucleic acid encoding the rAAV vector and nucleic acid encoding AAV rep and cap functions, and providing nucleic acid encoding AAV helper functions.

85. The rAAV particle of claim 84, wherein the AAV producer cell comprises nucleic acid encoding AAV helper functions.

86. The rAAV particle of claim 84, wherein the AAV helper functions are provided by infecting the AAV producer cells with an AAV helper virus that provides the AAV helper functions.

87. The rAAV particle of claim 86, wherein the AAV helper virus is an adenovirus, a herpes simplex virus, or a baculovirus.

88. The rAAV particle of any one of claims 80-87, wherein the AAV cap functions provide an amino acid substitution at amino acid residue 2 of VP1 and/or VP3, wherein the amino acid substitution at amino acid residue 2 of VP1 and/or VP3 alters N-terminal acetylation compared to N-terminal acetylation at amino acid residue 2 of VP1 and/or VP3 of the parent AAV particle.
89. A pharmaceutical composition comprising the rAAV particle of any one of claims 63-88.
90. A kit comprising the rAAV particle of any one of claims 63-87 or the pharmaceutical composition of claim 89.
91. An article of manufacture comprising the rAAV particle of any one of claims 63-87 or the pharmaceutical composition of claim 89.
92. An AAV capsid protein comprising an amino acid substitution at amino acid residue 2 of a parent AAV capsid protein; wherein the amino acid substitution at amino acid residue 2 alters N-terminal acetylation compared to N-terminal acetylation at amino acid residue 2 of the parent AAV capsid protein.
93. The AAV capsid protein of claim 92, wherein the substitution results in a higher frequency of N-terminal acetylation or a lower frequency of N-terminal acetylation.
94. The AAV capsid protein of claim 92 or 93, wherein the AAV capsid protein is VP1 or VP3.
95. The AAV capsid protein of any one of claims 92-94, wherein amino acid residue 2 is substituted with Cys, Ser, Thr, Val, Gly, Asn, Asp, Glu, Ile, Leu, Phe, Gln, Lys, Met, Pro or Tyr.

96. The AAV capsid protein of claim 93, wherein amino acid residue 2 is substituted with Ser, Asp or Glu.
97. The AAV capsid protein of any one of claims 92-96, wherein the amino acid substitution results in less deamidation of the AAV capsid.
98. The AAV capsid protein of any one of claims 92-97, comprising an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV LK03, AAV2R471A, AAV2/2-7m8, AAV DJ, an AAV DJ8 capsid, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV V708K, goat AAV, AAV1/AAV2 chimeric, bovine AAV, mouse AAV, rAAV2/HBoV1, AAV2HBKO, AAVPHP.B or AAVPHP.eB serotype capsid.
99. The rAAV capsid protein of any one of claims 92-98, wherein the AAV capsid protein further comprises a tyrosine mutation or a heparin binding mutation.
100. A method of improving the stability of a rAAV particle comprising substituting amino acid residue 2 of VP1 and/or VP3 of a parent VP1 and/or VP3; wherein the substituting the amino acid at position 2 alters N-terminal acetylation of VP1 and/or VP3, as compared to amino acid residue 2 of the parent VP1 and/or VP3.
101. A method of improving the assembly of rAAV particles in a cell comprising substituting amino acid residue 2 of VP1 and/or VP3; wherein the substituted amino acid at position 2 alters N-terminal acetylation of VP1 and/or VP3, as compared to amino acid residue 2 of the parent VP1 and/or VP3.

102. A method of improving transduction of rAAV particles in a cell comprising substituting amino acid residue 2 of VP1 and/or VP3 of a parent VP1 and/or VP3; wherein substituting the amino acid residue 2 alters N-terminal acetylation of VP1 and/or VP3, as compared to amino acid residue 2 of the parent VP1 and/or VP3.

103. The method of any one of claims 100-102, wherein substituting the amino acid residue 2 results in a higher frequency of N-terminal acetylation or a lower frequency of N-terminal acetylation.

104. The method of any one of claims 100-103, wherein amino acid residue 2 of VP1 is substituted.

105. The method of any one of claims 100-104, wherein amino acid residue 2 of VP3 is substituted.

106. The method of any one of claims 100-105, wherein amino acid residue 2 is substituted with Cys, Ser, Thr, Val, Gly, Asn, Asp, Glu, Ile, Leu, Phe, Gln, Lys, Met, Pro or Tyr.

107. The method of claim 106, wherein amino acid residue 2 is substituted with Ser, Asp or Glu.

108. The method of any one of claims 100-107, wherein the AAV particle comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV LK03, AAV2R471A, AAV2/2-7m8, AAV DJ, an AAV DJ8 capsid, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV V708K, goat AAV, AAV1/AAV2 chimeric, bovine AAV, mouse AAV, rAAV2/HBoV1, AAV2HBKO, AAVPHP.B, or AAVPHP.eB serotype capsid

109. The method of any one of claims 100-108, wherein the rAAV particle's capsid further comprises a tyrosine mutation or a heparin binding mutation.
110. The method of any one of claims 100-109, wherein the rAAV particle comprises a rAAV vector.
111. The method of claim 110, wherein the rAAV vector comprises one or more AAV ITRs.
112. The method of claim 110 or 111, wherein the rAAV vector comprises an AAV1 ITR, an AAV2 ITR, an AAV3 ITR, an AAV4 ITR, an AAV5 ITR, an AAV6 ITR, an AAV7 ITR, an AAV8 ITR, an AAVrh8 ITR, an AAV9 ITR, an AAV10 ITR, an AAVrh10 ITR, an AAV11 ITR, or an AAV12 ITR.
113. The method of any one of claims 110-112, wherein the AAV capsid and the AAV ITRs are derived from the same serotype.
114. The method of any one of claims 110-112, wherein the AAV capsid and the AAV ITRs are derived from different serotypes.
115. The method of any one of claims 100-114, wherein the AAV particle comprises an AAV vector encoding a heterologous transgene flanked by one or more AAV ITRs.
116. The method of any one of claims 110-115, wherein the rAAV vector is a self-complementary vector.

117. The method of claim 116, wherein the rAAV vector comprises first nucleic acid sequence encoding the transgene and a second nucleic acid sequence encoding a complement of the transgene, wherein the first nucleic acid sequence can form intrastrand base pairs with the second nucleic acid sequence along most or all of its length.

118. The method of claim 117, wherein the first nucleic acid sequence and the second nucleic acid sequence are linked by a mutated AAV ITR, wherein the mutated AAV ITR comprises a deletion of the D region and comprises a mutation of the terminal resolution sequence.

119. The method of any one of claims 100-118, wherein the rAAV particle is produced by transfecting a host cell with nucleic acid encoding the rAAV vector and nucleic acid encoding AAV rep and cap functions, and providing nucleic acid encoding AAV helper functions.

120. The method of claim 119, wherein the AAV helper functions are provided by transfecting the host cell with nucleic acid encoding the AAV helper functions.

121. The method of claim 120, wherein the AAV helper functions are provided by infecting the host cell with an AAV helper virus that provides the AAV helper functions.

122. The method of claim 121, wherein the AAV helper virus is an adenovirus, a herpes simplex virus or a baculovirus.

123. The method of any one of claims 100-118, wherein the rAAV particle is produced by an AAV producer cell comprising nucleic acid encoding the rAAV vector and nucleic acid encoding AAV rep and cap functions, and providing nucleic acid encoding AAV helper functions.

124. The method of claim 123, wherein the AAV producer cell comprises nucleic acid encoding AAV helper functions.

125. The method of claim 124, wherein the AAV helper functions are provided by infecting the AAV producer cells with an AAV helper virus that provides the AAV helper functions.

126. The method of claim 125, wherein the AAV helper virus is an adenovirus, a herpes simplex virus, or a baculovirus.

127. The method of any one of claims 119-126, wherein the AAV cap functions provide an amino acid substitution at amino acid residue 2 of VP1 and/or VP3, wherein the amino acid substitution at amino acid residue 2 of VP1 and/or VP3 alters N-terminal acetylation compared to N-terminal acetylation at amino acid residue 2 of VP1 and/or VP3 of the parent AAV particle.

128. A method to determine the serotype of a viral particle comprising

- a) denaturing the viral particle,
- b) subjecting the denatured viral particle to liquid chromatography/mass spectrometry (LC/MS), and
- c) determining the masses of one or more capsid proteins of the viral particle;

wherein the specific combination of masses of the one or more capsid proteins are indicative of the virus serotype.

129. The method of claim 128, wherein the calculated masses of the one or more capsid proteins are compared to the theoretical masses of the one or more capsid proteins of one or more virus serotypes.

130. A method of determining the heterogeneity of a viral particle comprising
- a) denaturing the viral particle,
 - b) subjecting the denatured viral particle to liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS),
 - c) determining the masses of one or more capsid proteins of the viral particle, and
 - d) comparing the masses of step c) with the theoretical masses of the one or more capsid proteins of the virus serotype;
- wherein a deviation of one or more of the masses of the one or more capsid proteins are indicative of the viral capsid heterogeneity.
131. The method of claim 130, wherein the heterogeneity comprises one or more of mixed serotypes, variant capsids, capsid amino acid substitutions, truncated capsids, or modified capsids.
132. The method of any one of claims 128-131, wherein the liquid chromatography is reverse phase liquid chromatography, size exclusion chromatography, hydrophilic interaction liquid chromatography, or cation exchange chromatography.
133. The method of any one of claims 128-132, wherein the viral particle comprises a viral vector encoding a heterologous transgene.
134. A method to determine the serotype of a viral particle comprising
- a) denaturing the viral particle,
 - b) subjecting the denatured viral particle to reduction and/or alkylation,
 - c) subjecting the denatured viral particle to digestion to generate fragments of one or more capsid proteins of the viral particle,

d) subjecting the fragments of the one or more capsid proteins to liquid chromatography/mass spectrometry-mass spectrometry (LC/MS/MS), and

e) determining the masses of fragments of the one or more capsid proteins of the viral particle;

wherein the specific combination of masses of fragments of the one or more capsid proteins are indicative of the viral serotype.

135. The method of claim 134, wherein the calculated masses of the fragments of the one or more capsid proteins are compared to the theoretical masses of fragments of the one or more capsid proteins of one or more viral serotypes.

136. A method of determining the heterogeneity of a serotype of a viral particle comprising

a) denaturing the viral particle,

b) subjecting the denatured viral particle to reduction and/or alkylation,

c) subjecting the denatured viral particle to digestion to generate fragments of one or more capsid proteins of the viral particle,

d) subjecting the fragments of the one or more capsid proteins to liquid chromatography/mass spectrometry-mass spectrometry (LC/MS/MS),

e) determining the masses of fragments of the one or more capsid proteins of the viral particle, and

f) comparing the masses of step e) with the theoretical masses of fragments of the one or more capsid proteins of the viral serotype;

wherein a deviation of one or more of the masses of the one or more capsid proteins are indicative of the viral capsid heterogeneity.

137. The method of claim 136, wherein the heterogeneity comprises one or more of mixed serotypes, variant capsids, capsid amino acid substitutions, truncated capsids, or modified capsids.

138. The method of any one of claims 134-137, wherein the liquid chromatography is reverse phase liquid chromatography, size exclusion chromatography, hydrophilic interaction liquid chromatography, or cation exchange chromatography.

139. The method of any one of claims 134-138, wherein the viral particle comprises a viral vector encoding a heterologous transgene.

140. The method of any one of claims 128-139, wherein the viral particle belongs to a viral family selected from the group consisting of *Adenoviridae*, *Parvoviridae*, *Retroviridae*, *Baculoviridae*, and *Herpesviridae*.

141. The method of claim 140, wherein the viral particle belongs to a viral genus selected from the group consisting of *Atadenovirus*, *Aviadenovirus*, *Ichtadenovirus*, *Mastadenovirus*, *Siadenovirus*, *Ambidensovirus*, *Brevidensovirus*, *Hepandensovirus*, *Iteradensovirus*, *Penstyldensovirus*, *Amdoparvovirus*, *Aveparvovirus*, *Bocaparvovirus*, *Copiparvovirus*, *Dependoparvovirus*, *Erythroparvovirus*, *Protoparvovirus*, *Tetraparvovirus*, *Alpharetrovirus*, *Betaretrovirus*, *Deltaretrovirus*, *Epsilonretrovirus*, *Gammaretrovirus*, *Lentivirus*, *Spumavirus*, *Alphabaculovirus*, *Betabaculovirus*, *Deltabaculovirus*, *Gammabaculovirus*, *Iltoivirus*, *Mardivirus*, *Simplexvirus*, *Varicellovirus*, *Cytomegalovirus*, *Muromegalovirus*, *Proboscivirus*, *Roseolovirus*, *Lymphocryptovirus*, *Macavirus*, *Percavirus*, and *Rhadinovirus*.

142. A recombinant AAV (rAAV) particle comprising one or more amino acid substitutions at amino acid residue A35, N57, G58, N382, G383, N511, G512, N715, or G716 of VP1 or VP3 of a parent AAV particle, wherein residue numbering is based on VP1 of AAV2 and the one or

more amino acid substitutions alters deamidation as compared to deamidation of VP1 and/or VP3 of the parent AAV particle.

143. The rAAV particle of claim 142, wherein the one or more amino acid substitutions is at amino acid residue A35 of VP1, N57 of VP1, G58 of VP1, N382 of VP3, G383 of VP3, N511 of VP3, G512 of VP3, N715 of VP3, or G716 of VP3 and alters deamidation as compared to deamidation of VP1 and/or VP3 of the parent AAV particle.

144. The rAAV particle of claim 142, wherein the one or more amino acid substitutions comprise a substitution of Asp at N57 of VP1, N382 of VP3, N511 of VP3, or N715 of VP3 and result in a higher frequency of deamidation as compared to deamidation of VP1 and/or VP3 of the parent AAV particle.

145. The rAAV particle of claim 142, wherein the one or more amino acid substitutions comprise a N57K or a N57Q substitution and results in a lower frequency of deamidation as compared to deamidation of VP1 and/or VP3 of the parent AAV particle.

146. The rAAV particle of claim 142, wherein the one or more amino acid substitutions comprise a substitution of Asn at A35 of VP1 and result in a higher frequency of deamidation as compared to deamidation of VP1 of the parent AAV particle.

147. The rAAV particle of claim 142, wherein the one or more amino acid substitutions is at G58 of VP1, G383 of VP3, G512 of VP3, or G716 of VP3 and result in a lower frequency of deamidation as compared to deamidation of VP1 and/or VP3 of the parent AAV particle.

148. The rAAV particle of claim 147, wherein the G58 of VP1 is substituted with Asp.

149. The rAAV particle of any one of claims 142-148, wherein the rAAV particle is an AAV1 particle or an AAV2 particle.

150. A pharmaceutical composition comprising the rAAV particle of any one of claims 142-149.

151. A kit comprising the rAAV particle of any one of claims 142-149 or the pharmaceutical composition of claim 150.

152. An article of manufacture comprising the rAAV particle of any one of claims 142-149 or the pharmaceutical composition of claim 150.

153. An AAV capsid protein comprising an amino acid substitution of a parent AAV capsid protein; wherein the amino acid substitution alters deamidation of the capsid compared to the parent AAV capsid protein.

154. A method of improving the stability of a rAAV particle comprising substituting one or more amino acid residues of VP1 and/or VP3 of a parent VP1 and/or VP3, wherein the one or more amino acid residues is A35, N57, G58, N382, G383, N511, G512, N715, or G716, residue numbering based on VP1 of AAV2; wherein the amino acid substitution alters deamidation as compared to amino acid residue 2 of the parent VP1 and/or VP3.

155. A method of improving the assembly of rAAV particles in a cell comprising substituting one or more amino acid residues of VP1 and/or VP3, wherein the one or more amino acid residues is A35, N57, G58, N382, G383, N511, G512, N715, or G716, residue numbering based

on VP1 of AAV2; wherein the amino acid substitution alters deamidation as compared to deamidation of VP1 and/or VP3 of the parent VP1 and/or VP3.

156. A method of improving the transduction of rAAV particles in a cell comprising substituting one or more amino acid residues, wherein the one or more amino acid residues is A35, N57, G58, N382, G383, N511, G512, N715, or G716, residue numbering based on VP1 of AAV2; wherein the amino acid substitution alters deamidation as compared to deamidation of VP1 and/or VP3 of the parent AAV particle.

157. The method of any one of claims 154-156, wherein the one or more amino acid substitutions is at A35 of VP1, N57 of VP1, G58 of VP1, N382 of VP3, G383 of VP3, N511 of VP3, G512 of VP3, N715 of VP3, or G716 of VP3; wherein the amino acid substitution alters deamidation as compared to deamidation of VP1 and/or VP3 of the parent AAV particle.

158. The method of any one of claims 154-157, wherein the parental Ala residue at position 35 of VP1 is substituted with Asn.

159. The method of any one of claims 154-158, wherein the parental Gly residue at position 58 of VP1 is substituted with Asp.

160. The method of any one of claims 154-159, wherein the AAV particle comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV LK03, AAV2R471A, AAV2/2-7m8, AAV DJ, an AAV DJ8 capsid, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV V708K, goat AAV, AAV1/AAV2 chimeric, bovine AAV, mouse AAV, rAAV2/HBoV1, AAV2HBKO, AAVPHP.B, or AAVPHP.eB serotype capsid

161. The method of any one of claims 154-160, wherein the rAAV particle's capsid further comprises a tyrosine mutation or a heparin binding mutation.
162. The method of any one of claims 154-160, wherein the rAAV particle is an AAV1 particle or an AAV2 particle.
163. The method of any one of claims 154-162, wherein the rAAV particle comprises a rAAV vector.
164. The method of claim 163, wherein the rAAV vector comprises one or more AAV ITRs.
165. The method of claim 163 or 164, wherein the rAAV vector comprises an AAV1 ITR, an AAV2 ITR, an AAV3 ITR, an AAV4 ITR, an AAV5 ITR, an AAV6 ITR, an AAV7 ITR, an AAV8 ITR, an AAVrh8 ITR, an AAV9 ITR, an AAV10 ITR, an AAVrh10 ITR, an AAV11 ITR, or an AAV12 ITR.
166. The method of any one of claims 164-165, wherein the AAV capsid and the AAV ITRs are derived from the same serotype.
167. The method of any one of claims 164-166, wherein the AAV capsid and the AAV ITRs are derived from different serotypes.
168. The method of any one of claims 164-167, wherein the AAV particle comprises an AAV vector encoding a heterologous transgene flanked by one or more AAV ITRs.
169. The method of any one of claims 164-168, wherein the rAAV vector is a self-complementary vector.

170. The method of claim 169, wherein the rAAV vector comprises first nucleic acid sequence encoding the transgene and a second nucleic acid sequence encoding a complement of the transgene, wherein the first nucleic acid sequence can form intrastrand base pairs with the second nucleic acid sequence along most or all of its length.

171. The method of claim 170, wherein the first nucleic acid sequence and the second nucleic acid sequence are linked by a mutated AAV ITR, wherein the mutated AAV ITR comprises a deletion of the D region and comprises a mutation of the terminal resolution sequence.

172. The method of any one of claims 164-171, wherein the rAAV particle is produced by transfecting a host cell with nucleic acid encoding the rAAV vector and nucleic acid encoding AAV rep and cap functions, and providing nucleic acid encoding AAV helper functions.

173. The method of claim 172, wherein the AAV helper functions are provided by transfecting the host cell with nucleic acid encoding the AAV helper functions.

174. The method of claim 173, wherein the AAV helper functions are provided by infecting the host cell with an AAV helper virus that provides the AAV helper functions.

175. The method of claim 174, wherein the AAV helper virus is an adenovirus, a herpes simplex virus or a baculovirus.

176. The method of any one of claims 164-175, wherein the rAAV particle is produced by an AAV producer cell comprising nucleic acid encoding the rAAV vector and nucleic acid encoding AAV rep and cap functions, and providing nucleic acid encoding AAV helper functions.

177. The method of claim 176, wherein the AAV producer cell comprises nucleic acid encoding AAV helper functions.

178. The method of claim 177, wherein the AAV helper functions are provided by infecting the AAV producer cells with an AAV helper virus that provides the AAV helper functions.

179. The method of claim 178, wherein the AAV helper virus is an adenovirus, a herpes simplex virus, or a baculovirus.

180. The method of any one of claims 172-179, wherein the AAV cap functions provide an amino acid substitution of VP1 and/or VP3, wherein the amino acid substitution of VP1 and/or VP3 alters deamidation compared to deamidation of VP1 and/or VP3 of the parent AAV particle.