CLAIMS

WE CLAIM:

1. A method to characterize VP1, VP2 and VP3 capsid proteins in an adeno-associated virus (AAV) particle, the method comprising:

subjecting the AAV particle to a liquid chromatography at about 70°C to about 90 °C;

wherein the ratio of VP1, VP2 and VP3 capsid proteins are determined by mass spectrometry and/or ultraviolet (UV)-visible spectroscopy.

2. The method of claim 1, wherein individual masses of VP1~~1~~, VP2 and VP3 capsid proteins are determined by the mass spectrometry.

3. The method of claim 1 or 2, wherein the ratio of the VP1, VP2 and VP3 capsid proteins is determined by comparing ultraviolet chromatogram (UV) of the VP1, VP2 and VP3 capsid proteins.

4. The method of any one of the preceding claims, wherein the liquid chromatography is a reverse phase liquid chromatography.

5. The method of claim 4, wherein the reverse phase liquid chromatography is performed using a C18 column, a C8 column, or a C4 column.

~~6. The method of claim 5, wherein the liquid chromatography is performed using a C8 column.~~

~~7~~6. The method of claim 5, wherein the column comprises particles of about 1.2-3.5 µm~~, or~~ optionally, wherein the column comprises particles of about 1.7 µm or about 1.8 µm.

~~8. The method of any one of claims 5-7, wherein the column comprises particles of about 1.7 µm or about 1.8 µm.~~

~~9~~7. The method of any one of claims ~~5-8~~5 and 6, wherein the column is from about 50 mm to about 300 mm long and has an internal diameter of from about 1 mm to about 4.6 mm.

~~10. The method of claim 9, wherein the chromatography column that is about 100 mm long and has an internal diameter of about 2.1 mm.~~

~~11~~8. The method of any one of claims 1-~~10~~7, wherein the AAV particle is of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV 13, AAVrh10, AAVrh74 serotype, or any naturally occurring, recombinant, or synthetic AAV particle.

~~12. The method of any one of claims 1-11, wherein the AAV particle is of AAVrh74 serotype.~~

~~13~~9. The method of any one of claims 1-~~12~~8, wherein the liquid chromatography is performed at about 75°C to about 85 °C.

~~14. The method of any one of claims 1-13, wherein the liquid chromatography is performed at about 80 °C.~~

~~15~~9. The method of any one of claims 1-~~14~~8, wherein the liquid chromatography comprises a first mobile phase that comprises trifluoroacetic acid, optionally wherein the first mobile phase comprises from about 0.05 to about 0.15% trifluoroacetic acid by volume.

~~16. The method of claim 15, wherein the first mobile phase comprises from about 0.05 to about 0.15% trifluoroacetic acid by volume.~~

~~17. The method of claim 15 or 16, wherein the first mobile phase comprises about 0.1% trifluoroacetic acid by volume.~~

~~18~~10. The method of any one of the preceding claims, wherein the liquid chromatography comprises a second mobile phase that comprises trifluoroacetic acid in the mixture of acetonitrile and water, optionally wherein the second mobile phase comprises from about 0.05 to about 0.15% trifluoroacetic acid by volume.

~~19. The method of claim 18, wherein the second mobile phase comprises from about 0.05 to about 0.15% trifluoroacetic acid by volume.~~

~~20. The method of claim 18 or 19, wherein the second mobile phase comprises about 0.1% trifluoroacetic acid by volume.~~

~~21~~11. The method of ~~any one of claims 18-20~~claim 10, wherein the second mobile phase comprises about 80-95% of acetonitrile by volume, optionally wherein the second mobile phase comprises about 90% of acetonitrile and 10% water by volume.

~~22. The method of any one of claims 18-21, wherein the second mobile phase comprises about 90% of acetonitrile and 10% water by volume.~~

~~23~~12. The method of ~~any one of claims 18-22~~claim 10 or 11, wherein the percentage of the second mobile phase, as compared to a combination of the first mobile phase and the second mobile phase, in the liquid chromatography is increased over time.

~~24~~13. The method of claim ~~23~~12, wherein the percentage of the second mobile phase is increased from about 10% to about 40%, and then from about 40% to about 45%, by volume, optionally wherein the percentage of the second mobile phase is increased: from about 10% to about 40%, by volume, over about 3 min; and then from about 40% to about 45%, by volume, over about 30 min.

~~25. The method of claim 24, wherein the percentage of the second mobile phase is increased: from about 10% to about 40%, by volume, over about 3 min; and then from about 40% to about 45%, by volume, over about 30 min.~~

~~26~~14. The method of claim ~~25~~13, wherein the percentage of the second mobile phase, in the combination of the first mobile phase and the second mobile phase, is subsequently increased to 100%, by volume, over about 1 min, optionally wherein the percentage of the second mobile phase, in the ~~comparison~~combination of the first mobile phase and the second mobile phase, is subsequently decreased to 10%, by volume, over about 1 min.

~~27. The method of claim 26, wherein the percentage of the second mobile phase, in the combination of the first mobile phase and the second mobile phase, is subsequently decreased to 10%, by volume, over about 1 min.~~

~~28~~15. The method of any one of the preceding claims, wherein the mass spectrometry is performed using a fragmentor voltage of about 125-350 V.

~~29. The method of any one of the preceding claims, wherein the mass spectrometry is performed using a fragmentor voltage of about 175 V.~~

~~30~~16. The method of any one of the preceding claims, wherein the mass spectrometry is performed using a capillary voltage of about 3-6 kV.

~~31. The method of any one of the preceding claims, wherein the mass spectrometry is performed using a capillary voltage of about 5 kV.~~

~~32~~17. The method of claim 1, wherein the characterization further comprises determining a post translational modification of at least one of VP1~~1~~, VP2 and VP3 capsid proteins.

~~33~~18. The method of claim ~~32~~17, wherein the post translational modification comprises one or more of loss of amino acid, glycosylation, sialylation, acetylation, phosphorylation, deamidation, oxidation, formylation, hydroxylation, methylation, and sulfation, optionally wherein the post translational modification comprises one or more of loss of N-terminal methionine, deamidation, loss of threonine, phosphorylation, and acetylation. .

~~34. The method of claim 32, wherein the post translational modification comprises one or more of loss of N-terminal methionine, deamidation, loss of threonine, phosphorylation, and acetylation.~~

~~35~~19. The method of any one of claims 1-~~33~~18, wherein the method is performed with a gradient program listed in Table 2.

~~36~~20. The method of claim ~~32~~17, wherein the characterization uses a buffer comprising Tris-HCl, optionally acetonitrile, and/or optionally methionine.

~~37. The method of claim 36, wherein the buffer comprises acetonitrile.~~

~~38. The method of claim 36, wherein the buffer comprises methionine.~~

~~39~~21. The method of ~~any one of claims 36-38~~claim 20, wherein the buffer comprises Tris-HCl at 5mM to 50 mM, 5%-20% acetonitrile, and methionine at 1 mM to 50 mM, optionally wherein the buffer comprises Tris-HCl at 20 mM, 5%-10% acetonitrile, and methionine at 10 mM.

~~40. The method of claim 39, wherein the buffer comprises Tris-HCl at 20 mM, 5%-10% acetonitrile, and methionine at 10 mM.~~

~~41~~22. The method of claim ~~36~~20, wherein the T~~t~~ris-HCl is at pH 7.5.

~~42~~23. The method of claim ~~32~~17, wherein the post-translation modification comprises deamidation at one or more of N263, N514, N57, N502, N254, and N94 of AAV8 or its equivalent residue at AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV9, AAV10, AAV11, AAV12, AAV 13, AAVrh10, ~~or~~ AAVrh74, or a recombinant or synthetic AAV particle.

~~43. The method of claim 32, wherein the post-translation modification comprises deamidation at one or more of N57, N255, N256, and N263 of AAV. Rh74.~~

~~44. The method of claim 32, wherein the post-translation modification comprises oxidation at one or more of M437, M473, M526, M544, M560, and M637 of AAV. Rh74.~~

~~45~~24. An AAV composition comprising an AAV capsid that comprises deamidation at one or more of N57, N255, N256, and N263 of AAV. Rh74 or the equivalent residues of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV 13, ~~or~~ AAVrh10, or a recombinant or synthetic AAV particle.

~~46~~25. An AAV composition comprising an AAV capsid that comprises oxidation at one or more of M437, M473, M526, M544, M560, and M637 of AAV. Rh74 or the equivalent residues of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, CAAV11, AAV12, AAV 13, ~~or~~ AAVrh10, or a recombinant or synthetic AAV particle.

~~47. A method of characterizing host cell proteins in an AAV composition, comprising:~~

~~immunoprecipitating viral capsid proteins from the composition;~~

~~digesting residual host cell proteins;~~

~~analyzing the digested proteins with liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS) to identify host cell proteins.~~

~~48. The method of claim 47, wherein the immunoprecipitation comprises incubating the AAV composition with an anti-AAV VP1 antibody, an anti-AAV VP2 antibody, an anti-AAV VP3 antibody, or combinations thereof.~~

~~49. The method of claim 47 or 48, further comprising analyzing the digested host cell proteins with iterative MS/MS.~~

~~50. The method of any one of claims 47-49, wherein the digestion is done in solution.~~

~~51. The method of claim 50, wherein the digestion comprises rapid digestion performed at a temperature of about 60°C to about 80°C.~~

~~52. The method of claim 51, wherein the temperature is about 70°C.~~

~~53. The method of any one of claims 47-52, further comprising spiking the AAV composition with a known amount of at least one known protein standard.~~

~~54. The method of claim 53, wherein the at least one known protein standard is a human or bovine protein standard.~~

~~55. The method of claims 53 or 54, further comprising quantifying the amount of the digested host cell proteins relative to the at least one protein standard.~~

~~56. The method of any one of claims 47-55, wherein the liquid chromatography is a reverse phase liquid chromatography.~~

~~57. The method of claim 56, wherein the reverse phase liquid chromatography is performed using a C18 column, a C8 column, or a C4 column.~~

~~58. The method of claim 57, wherein the liquid chromatography is performed using a C8 column.~~

~~59. The method of claim 57 or 58, wherein the column comprises particles of about 1.2-3.5 µm.~~

~~60. The method of any one of claims 57-59, wherein the column comprises particles of about 1.7 µm or about 1.8 µm.~~

~~61. The method of any one of claims 57-60, wherein the column is from about 50 mm to about 300 mm long and has an internal diameter of from about 1 mm to about 4.6 mm.~~

~~62. The method of claim 61, wherein the column is about 150 mm long and has an internal diameter of about 2.1 mm.~~

~~63. The method of any one of claims 47-62, wherein the liquid chromatography is performed at about 40°C to about 50°C.~~

~~64. The method of any one of claims 47-63, wherein the liquid chromatography is performed at about 45°C.~~

~~65. The method of any one of claims 47-64, wherein the liquid chromatography comprises a first mobile phase that comprises formic acid.~~

~~66. The method of claim 65, wherein the first mobile phase comprises from about 0.05% to about 0.15% formic acid by volume.~~

~~67. The method of claim 65 or 66, wherein the first mobile phase comprises about 0,1% formic acid by volume.~~

~~68. The method of any one of claims 47-67, wherein the liquid chromatography comprises a second mobile phase that comprises formic acid in a mixture of acetonitrile and water.~~

~~69. The method of claim 68, wherein the second mobile phase comprises from about~~

~~0.05% to about 0.15% formic acid by volume.~~

~~70. The method of claim 68 or 69, wherein the second mobile phase comprises about 0.1% formic acid by volume.~~

~~71. The method of any one of claims 68-70, wherein the second mobile phase comprises about 80-95% acetonitrile by volume.~~

~~72. The method of claim 71, wherein the second mobile phase comprises about 90% acetonitrile by volume and about 10% water by volume.~~

~~73. The method of any one of claims 68-72, wherein the percentage of the second mobile phase, as compared to a combination of the first mobile phase and the second mobile phase, in the liquid chromatography is increased over time.~~

~~74. The method of claim 73, wherein the percentage of the second mobile phase is increased from about 2% to about 50%.~~

~~75. The method of claim 74, wherein the percentage of the second mobile phase is increased from about 2% to about 50% by volume over about 120 minutes.~~

~~76. The method of claim 75, wherein the percentage of the second mobile phase is subsequently increased to 100% by volume for about 5 minutes.~~

~~77. The method of claim 76, wherein the percentage of the second mobile phase is subsequently maintained at 100% by volume for about 3 minutes.~~

~~78. The method of claim 77, wherein the percentage of the second mobile phase is subsequently decreased to about 2% by volume for about 2 minutes.~~

~~79. The method of any one of claims 47-78, wherein the mass spectrometry is performed using a fragmentor voltage of about 125-350 V.~~

~~80. The method of any one of claims 47-79, wherein the mass spectrometry is performed using a fragmentor voltage of about 135V.~~

~~81. The method of any one of claims 47-80, wherein the mass spectrometry is performed using a capillary voltage of about 3-6 kV.~~

~~82. The method of any one of claims 47-81, wherein the mass spectrometry is performed using a capillary voltage of about 4 kV.~~

~~83. A recombinant AAV (rAAV) comprising a heterogeneous group of capsid proteins that contain a subpopulation with an amino acid modification, wherein the amino acid modification is a post-translation modification.~~

~~84. The rAAV of claim 83, wherein the modification is deamidation or oxidation.~~

~~85. The rAAV of claims 83 or 84, wherein the heterogeneous group comprises a deamidated asparagine (N) at one or more of N57, N255, N256, and N263 of AAV. rh74 or the equivalent residues of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV 13, or AAVrh10, as measured by mass spectrometry and/or ultraviolet (UV) visible spectroscopy.~~

~~86. The rAAV of claim 85, wherein the heterogeneous group comprises less than 70%, 60%, 50%, 40%, 30%, 20%, or 15% of capsid proteins with deamidation at N57 of AAV. rh74 capsid.~~

~~87. The rAAV of claim 85, wherein the heterogeneous group comprises less than 15% of capsid proteins with deamidation at N57 of AAV. rh74 capsid.~~

~~88. The rAAV of claim 85, wherein the heterogeneous group comprises less than 70%, 60%, 50%, 40%, 30%, 20%, 15%, 10%, or 5% of capsid proteins with deamidation at N254 and/or N255 of AAV. rh74 capsid.~~

~~89. The rAAV of claim 85, wherein the heterogeneous group comprises less than 70%, 60%, 50%, 40%, 30%, 20% of capsid proteins with deamidation at N263.~~

~~90. The rAAV of claims 83 or 84, wherein the heterogeneous group comprises an oxidized methionine at one or more of M437, M473, M526, M544, M560, and M637 of AAV.~~

~~Rh74 or the equivalent residues of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, CAAV11, AAV12, AAV 13, or AAVrh10, as measured by mass spectrometry and/or ultraviolet (UV) visible spectroscopy.~~

~~91. The rAAV of claim 90, wherein the heterogeneous group comprises less than 30%, 20%, 10%, 5%, or 1% of capsid proteins with oxidation at M437.~~

~~92. The rAAV of claim 90, wherein the heterogeneous group comprises less than 30%, 20%, 10%, 5%, or 1% of capsid proteins with oxidation at M473.~~

~~93. The rAAV of claim 90, wherein the heterogeneous group comprises less than 30%, 20%, 10%, 5%, or 3% of capsid proteins with oxidation at M526.~~

~~94. The rAAV of claim 90, wherein the heterogeneous group comprises less than 30%, 20%, 10%, 5%, or 2% of capsid proteins with oxidation at M544.~~

~~95. The rAAV of claim 90, wherein the heterogeneous group comprises less than 30%, 20%, 10%, 5%, or 2% of capsid proteins with oxidation at M560.~~

~~96. The rAAV of claim 90, wherein the heterogeneous group comprises less than 30%, 20%, 10%, 5%, or 1% of capsid proteins with oxidation at M637.~~

~~97. The rAAV of any one of claims 83-85, wherein the heterogeneous group comprises a deamidated asparagine (N) within a peptide sequence of any one of SEQ ID Nos: 1-5, or its equivalent peptide sequence of other AAV serotype.~~