

CLAIMS

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,
optionally 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.
2. 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,
optionally wherein the heterogeneity comprises one or more of mixed serotypes, variant capsids, capsid amino acid substitutions, truncated capsids, or modified capsids.
3. The method of any one of claims 1-2, wherein the AAV particle is denatured with acetic acid, guanidine hydrochloride and/or an organic solvent.
4. The method of any one of claims 1-3, wherein the liquid chromatography is reverse phase liquid chromatography, size exclusion chromatography, hydrophilic interaction liquid chromatography, or cation exchange chromatography.
5. The method of claim 4, wherein the reverse phase chromatography is a C4 or C8 reverse chromatography.
6. The method of claim 5, wherein the chromatography uses a mobile phase A comprising formic acid in water, optionally wherein the mobile phase A comprises about 0.1% formic acid.

7. The method of claim 5 or claim 6, wherein the chromatography comprises a mobile phase B comprising formic acid in acetonitrile, optionally wherein the mobile phase B comprises about 0.1% formic acid.
8. The method of claim 7, wherein the proportion of mobile phase B in the chromatography increases over time,
optionally wherein the proportion of mobile phase B in the chromatography increases in a stepwise manner and wherein mobile phase B increases from about 10% to about 20%, from about 20% to about 30%, and from about 30% to about 38%,
optionally 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.
9. The method of any one of claims 1-8, wherein the liquid chromatography is ultra-performance liquid chromatography (UPLC).
10. The method of any one of claims 1-9, wherein the mass spectrometry comprises:
 - (i) a capillary voltage of about 3.5 kV;
 - (ii) a sampling cone voltage of about 45 V; and/or
 - (iii) assisted calibration, optionally wherein sodium iodide is used as a calibrant.
11. The method of any one of claims 1-10, wherein the N-terminus of VP1 and/or VP3 is acetylated.
12. The method of any one of claims 1-11, wherein the AAV particle:
 - (i) is a recombinant AAV (rAAV) particle; and/or
 - (ii) 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,
optionally wherein the AAV capsid comprises a tyrosine mutation or a heparin binding mutation.

13. The method of any one of claims 1-12, wherein:
- (i) 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; and/or
 - (ii) 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 AAV 10 ITR, an AAVrh10 ITR, an AAV11 ITR, or an AAV12 ITR.
14. The method of any one of claims 1-13, wherein the AAV particle comprises an AAV vector encoding a heterologous transgene.
15. A method of determining the serotype of an AAV particle comprising the method of any one of claims 1, or 3-14 combined with a further 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.
16. A method of determining heterogeneity of an AAV particle comprising the method of any one of claims 2-14 combined with a further 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.