CLAIMS

What is claimed is:

1. A method of delivering a cargo to an eye of a subject, the method comprising administering to the eye of the subject a rAAV particle comprising: (a) a capsid that is admixed with hyaluronic acid (HA) and (b) a cargo, wherein the cargo is delivered to the eye.

2. The method of claim 1, wherein the capsid comprises one or more surface-exposed patches of positively-charged amino residues.

3. The method of claim 1 or 2, wherein the serotype of the capsid is AAV2 or a variant thereof.

4. The method of any one of claims 1-3, wherein the capsid comprises non-native amino acid substitutions at amino acid residues of a wild-type AAV2 capsid as set forth in SEQ ID NO: 2, wherein the non-native amino acid substitutions comprise one or more of Y272F, Y444F, T491V, Y500F, Y700F, Y704F or Y730F.

5. The method of any one of claims 1-3, wherein the capsid is an AAV2G9 capsid.

6. The method of claim 1 or 2, wherein the capsid comprises non-native amino acid substitutions of a wild-type AAV2 capsid as set forth in SEQ ID NO: 2, wherein the non-native amino acid substitutions comprise:

   (a) Y444F;
   (b) Y444F+Y500F+Y730F;
   (c) Y272F+Y444F+Y500F+Y730F;
   (d) Y444F+Y500F+Y730F+T491V; or
   (e) Y272F+Y444F+Y500F+Y730F+T491V, or at equivalent amino acid positions corresponding thereto in any one of the wild-type AAV1, AAV3, AAV4, AAV5, AAV7, AAV9, or AAV10 capsid proteins, as set forth, respectively, in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, or SEQ ID NO: 10.

7. The method of claim 1 or 2, wherein the serotype of the capsid is AAV6 or a variant thereof.

8. The method of any one of claims 1, 2 and 7, wherein the capsid comprises non-native amino acid substitutions at amino acid residues of a wild-type AAV6 capsid as set forth in SEQ ID NO: 6, wherein the non-native amino acid substitutions comprise one or more of Y445F, Y705F, Y731F, T492V and S663V.

9. The method of any one of claims 1-3 and 7, wherein the capsid comprises non-native amino acid substitutions of a wild-type AAV6 capsid as set forth in SEQ ID NO: 6, wherein the non-native amino acid substitutions comprise:

(a) Y445F;
(b) Y705F+Y731F;
(c) T492V;
(d) Y705F+Y731F+T492V;
(e) S663V; or
(f) S663V+T492V.

10. The method of claim 1 or 2, wherein the capsid is selected from the group consisting of a AAV7m8 capsid, a AAV-DJ capsid, a AAV2/2-MAX capsid, a AAVSHh10 capsid, and a AAVSHh10Y capsid.

11. The method of claim 1 or 2, wherein the capsid is selected from the group consisting of a AAV3 capsid, a AAV3b capsid, and a AAVLK03 capsid.

12. The method of claim 1 or 2, wherein the capsid comprises a non-native amino acid substitution at amino acid residue 531 of a wild-type AAV1 capsid as set forth in SEQ ID NO: 1, wherein the non-native amino acid substitution is E531K.

13. The method of claim 1 or 2, wherein the capsid is AAV7BP2 capsid.

14. The method of any claim 1 or 2, wherein the capsid comprises non-native amino acid substitutions at amino acid residues 533 and/or 733 of a wild-type AAV8 capsid as set forth in SEQ ID NO: 8, wherein the non-native amino acid substitution is E533K and/or Y733F.

15. The method of any one of claims 1-14, wherein the capsid is pre-incubated with the HA prior to the step of administering.

16. The method of any one of claims 1-15, wherein the capsid is pre-incubated with a buffer that comprises the HA.

17. The method of any one of claims 15-16, wherein the capsid is pre-incubated with the HA for a duration of about 5 minutes, about 15 minutes, about 30 minutes, about 45 minutes, about 60 minutes or about 75 minutes.

18. The method of claim 17, wherein the capsid is pre-incubated for a duration of about 15 minutes.

19. The method of any one of claims 15-18, wherein the capsid is pre-incubated with the HA in a concentration of 0.1%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.75%, or 1.0% weight by volume.

20. The method of any one of claims 1-19, wherein the rAAV particle is administered to the eye of the subject in a titer of about $1x10^{10}$ vg/ml, $5 x10^{10}$ vg/ml, $1x10^{11}$ vg/ml, $5 x10^{11}$ vg/ml, $1x10^{12}$ vg/ml, $2x10^{12}$ vg/ml, $3x10^{12}$ vg/ml, $4x10^{12}$ vg/ml, about $5x10^{12}$ vg/ml, about $1x10^{13}$ vg/ml, or about $5x10^{13}$ vg/ml.

21. The method of any one of claims 1-20, wherein the rAAV particle is administered to the eye of the subject in a titer of less than $5 x10^{11}$ vg/ml.

22. The method of any one of claims 1-22, wherein the rAAV particle is administered by intravitreal injection.

23. The method of claim 22, wherein the intravitreal injection is provided in a volume of about 200 µL, about 175 µL, about 160 µL, about 145 µL, about 130 µL, about 115 µL, about 100 µL, about 90 µL, about 80 µL, about 70 µL, about 60 µL, about 55 µL, about 50 µL, about 45 µL, about 35 µL, about 20 µL, about 10 µL, or about 5 µL.

24. The method of claim 23, wherein the intravitreal injection is administered in a volume of about 50 µL.

25. The method of any one of claims 1-24, wherein the cargo comprises a polynucleotide comprising a heterologous nucleic acid sequence.

26. The method of claim 25, wherein the heterologous nucleic acid sequence is operably linked to a regulatory sequence that direct expression of the heterologous nucleic acid sequence in a photoreceptor cell, retinal pigment epithelium cell, retinal ganglion cell, bipolar cell, Müller glial cell or astrocyte cell.

27. The method of claim 26, wherein the regulatory sequence is selected from the group consisting of: a woodchuck hepatitis virus post-transcription regulatory element (WPRE), a polyadenylation signal sequence, an intron/exon junctions/splicing signal, and any combination thereof.

28. The method of any one of claims 25-27, wherein the heterologous nucleic acid sequence encodes a therapeutic agent.

29. The method of claim 28, wherein the therapeutic agent is a neurotrophic factor or an optogenetic actuator.

30. The method of claim 29, wherein the therapeutic agent is the neurotrophic factor.

31. The method of claim 30, wherein the neurotrophic factor is selected from the group consisting of: brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3, ciliary neurotrophic factor (CNTF), an ephrin, glial cell line-derived neurotrophic factors (GDNF), and a combination thereof.

32. The method of claim 29, wherein the therapeutic agent is the optogenetic actuator.

33. The method of claim 32, wherein the optogenetic actuator is selected from the group consisting of: a bacteriorhodopsin, a halorhodopsin, a channelrhodopsin, a microbial sensory rhodopsin, a mammalian rhodopsin, a cone opsin, a melanopsin, and a combination thereof.

34. The method of any one of claims 28-33, wherein the subject is in need of a therapeutically effective amount of the therapeutic agent.

35. The method of any one of claims 28-34, wherein the a rAAV particle is administered for a time effective to provide a therapeutically-effective amount of the therapeutic agent.

36. A method for treating or ameliorating one or more symptoms of a disease, disorder or condition, the method comprising intravitreally administering to the subject a rAAV particle comprising a capsid admixed with hyaluronic acid (HA) to an eye of a subject for a time sufficient to treat or ameliorate the one or more symptoms of the disease, disorder or condition in the subject, wherein the rAAV particle comprises (a) a polynucleotide encoding a therapeutic agent and (b) the serotype of the capsid is AAV2 or AAV6.

37. A method for providing a mammal in need thereof with a therapeutically effective amount of a therapeutic agent, the method comprising intravitreally administering to the subject a rAAV particle comprising a capsid that is admixed with hyaluronic acid (HA) to an eye of the subject for a time effective to provide the subject with a therapeutically-effective amount of the therapeutic agent, wherein the rAAV particle comprises an AAV7m8, an AAV-DJ, an AAV2/2-MAX, an AAVSHh10, an AAVSHh10Y, an AAV3, an AAV3b, or an AAVLK03 capsid.

38. A method for treating or ameliorating one or more symptoms of a disease, disorder or condition, the method comprising intravitreally administering to the subject a rAAV particle comprising a capsid that is admixed with hyaluronic acid (HA) to an eye of the subject in need thereof for a time sufficient to treat or ameliorate the one or more symptoms of the disease, disorder or condition in the subject, wherein the rAAV particle comprises (a) a polynucleotide encoding a therapeutic agent and (a) an AAV7m8, an AAV-DJ, an AAV2/2-MAX, an AAVSHh10, an AAVSHh10Y, an AAV3, an AAV3b, or an AAVLK03 capsid.

39. The method of any one of claims 36-38, wherein the disease, disorder or condition is age-related macular degeneration (AMD), wet AMD, dry AMD, or geographic atrophy.

40. The method of any one of claims 36-39, wherein the subject is a human.

41. The method of any one of claims 36-40, wherein production of the therapeutic agent a) preserves one or more photoreceptor cells or one or more RPE cells, b) restores one or more rod- and/or cone-mediated functions, c) restores visual behavior in one or both eyes, or d) any combination thereof.

42. The method of claim 41, wherein production of the therapeutic agent persists in the one or more photoreceptor cells or the one or more RPE cells substantially for a period of at least three months following an initial administration of the rAAV particle into the one or both eyes of the mammal.

43. The method of any one of claims 36-40, wherein production of the therapeutic agent preserves one or more retinal ganglion cells, Müller glial cells or astrocyte cells.

44. The method of claim 43, wherein the therapeutic agent comprises a neurotrophic factor.

45. The method of claim 43 or 44, wherein the disease, disorder or condition is retinitis pigmentosa or glaucoma.

46. The method of any one of claims 36-40, wherein production of the therapeutic agent preserves one or more retinal ganglion cells or retinal bipolar cells.

47. The method of claim 46, wherein the therapeutic agent comprises an optogenetic actuator.

48. The method of claim 47, wherein the optogenetic actuator is selected from the group consisting of: a bacteriorhodopsin, a halorhodopsin, a channelrhodopsin, a microbial sensory rhodopsin, a mammalian rhodopsin, a cone opsin, a melanopsin, and a combination thereof.

49. A buffer for storing a mixture of AAV and hyaluronic acid (HA), comprising:

(a) HA in a concentration of 0.1%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.75%, or 1.0% weight by volume;
(b) balanced salt solution (BSS);
(c) artificial cerebrospinal fluid; and
(d) phosphate buffered saline (PBS).

50. The buffer of claim 49 further comprising:

a. Ringer's lactate solution;
b. TMN200 solution;

c. Polysorbate 20;
d. poloxamer 188; or
e. a combination thereof.

51. The buffer of claim 49 or 50, wherein the HA is in a concentration of 0.4% weight by volume.

CLAIMS

1. A composition for use in delivering a cargo to an eye of a subject in need thereof, the composition comprising an rAAV particle comprising: (a) a capsid that is admixed with hyaluronic acid (HA) and (b) a cargo, wherein the rAAV particle is to be administered intravitreally, whereby the cargo is to be delivered to the eye.

2. The composition for use of claim 1, wherein the capsid comprises one or more surface-exposed patches of positively-charged residues.

3. The composition for use of claim 1 or 2, wherein the rAAV particle comprises a capsid selected from AAV2, AAV2(Y444F), AAV2(trpYF), AAV2 (quadYF), AAV2(quadYF+T-V), AAV2/2-MAX, DGE-DF, ME-B, ME-B(Y-F+T-V), P2-V2, P2-V3, AAV6, AAV7m8, AAV-DJ, AAV3, AAV3b, AAV6-3pMut, AAVSHh10, AAVSHh10Y, and AAVLK03.

4. The composition for use of any one of claims 1-3, wherein the use further comprises pre-incubating the capsid with the HA prior to administration of the rAAV particle.

5. The composition for use of any one of claims 1-4, wherein the capsid is pre-incubated with a buffer that comprises the HA.

6. The composition for use of claim 5, wherein the buffer comprises the HA in a concentration of 0.1%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.6%, 0.75%, or 1.0% weight by volume.

7. The composition for use of any one of claims 1-6, wherein the rAAV particle is administered to the eye of the subject in a titer of about 1×10^{10} vg/ml, about 5×10^{10} vg/ml, about 1×10^{11} vg/ml, about 5×10^{11} vg/ml, about 1×10^{12} vg/ml, about 2×10^{12} vg/ml, about 3×10^{12} vg/ml, about 4×10^{12} vg/ml, about 5×10^{12} vg/ml, about 1×1013 vg/ml, or about 5×10^{13} vg/ml.

8. The composition for use of any one of claims 1-7, wherein the cargo comprises a polynucleotide comprising a heterologous nucleic acid sequence.

9. The composition for use of claim 8, wherein the heterologous nucleic acid sequence is operably linked to a regulatory sequence that directs expression of the heterologous nucleic acid sequence in a photoreceptor cell, retinal pigment epithelium cell, retinal ganglion cell, bipolar cell, Müller glial cell or astrocyte cell.

10. The composition for use of claim 9, wherein the regulatory sequence is selected from the group consisting of: a woodchuck hepatitis virus post-transcription regulatory element (WPRE), a polyadenylation signal sequence, an intron/exon junctions/splicing signal, and a combination thereof.

11. The composition for use of claim 8, wherein the heterologous nucleic acid sequence encodes a therapeutic agent.

12. The composition for use of claim 11, wherein the therapeutic agent is:

    (a) a neurotrophic factor selected from the group consisting of: brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3, ciliary neurotrophic factor (CNTF), an ephrin, glial cell line-derived neurotrophic factor (GDNF), and a combination thereof; or

    (b) an optogenetic actuator selected from the group consisting of: a bacteriorhodopsin, a halorhodopsin, a channelrhodopsin, a microbial sensory rhodopsin, a mammalian rhodopsin, a cone opsin, a melanopsin, and a combination thereof.

13. The composition for use of any one of claims 1-12, wherein the composition is for use in treating a disease or disorder selected from the group consisting of: retinitis pigmentosa, Leber Congenital Amaurosis, age related macular degeneration (AMD), wet AMD, dry AMD, uveitis, Best disease, Stargardt's disease, Usher Syndrome, geographic atrophy, diabetic retinopathy, retinoschisis, achromatopsia, choroideremia, Bardet-Biedl syndrome, a glycogen storage disease, and a combination thereof.

14. The composition for use of any one of claims 1-13, wherein transduction is improved through a CD44-independent mechanism.

15. A composition comprising a rAAV particle comprising: (a) a capsid admixed with hyaluronic acid and (b) a cargo, wherein the hyaluronic acid has an average molecular weight of 4 kDa to 5,000 kDa.

16. The composition of claim 15, wherein the hyaluronic acid has an average molecular weight of 20 kDa to 5,000 kDa.

17. The composition of claim 15 or 16, wherein the hyaluronic acid is present at a concentration of 0.1% to 1.0% weight by volume.

18. The composition of any one of claims 15-18, wherein the composition is formulated for intravitreal injection.

19. The composition of claim 18, wherein the composition is formulated with one or more excipients from the group comprising balanced salt solution, Tween20, poloxamer 188, and TMN200.

20. The composition of any one of claims 15-19, wherein the cargo comprises a polynucleotide encoding a therapeutic agent.

21. The composition of any one of claims 15-20 for use as a medicament.

22. Use of an rAAV particle comprising: (a) a capsid that is admixed with hyaluronic acid (HA) and (b) a cargo, in the preparation of a composition for delivering a cargo to an eye of a subject in need thereof, wherein the rAAV particle is to be administered intravitreally.

23. Use of the composition of any one of claims 1-21 in the preparation of a medicament for delivering a cargo to an eye of a subject in need thereof, wherein the rAAV particle is to be administered intravitreally.

24. The use of any one of claims 22-23, wherein the composition or medicament is for treating a disease or disorder selected from the group consisting of: retinitis pigmentosa, Leber Congenital Amaurosis, age related macular degeneration (AMD), wet AMD, dry AMD, uveitis, Best disease, Stargardt’s disease, Usher Syndrome, geographic atrophy, diabetic retinopathy, retinoschisis, achromatopsia, choroideremia, Bardet-Biedl syndrome, a glycogen storage disease, and a combination thereof.