Furthermore, in gene therapy, certain clinical scenarios involving gene silencing or tissue degeneration may require multiple AAV vector administrations to sustain long term expression of the transgene. Compositions and methods to increase the efficiency of gene therapy involving rAAV would therefore be broadly useful. To facilitate effective clinical use, recombinant AAV vectors which evade antibody recognition (AAVe) are required. Such AAV vectors will help a) expand the eligible cohort of patients suitable for AAV-based gene therapy and b) allow multiple, repeat administrations of AAV-based gene therapy vectors.

Provided herein are methods and compositions comprising an adeno-associated virus (AAV) capsid protein that overcome previous shortcomings in the art. The AAV capsid proteins comprise one or more amino acid substitutions, wherein the substitutions introduce into an AAV vector comprising these modified capsid proteins the ability to evade host antibodies.

SUMMARY

Despite recent success in gene delivery with rAAV particles, pre-existing antibodies, which are able to neutralize rAAV particles administered to a subject, represent a significant challenge. Compositions of rAAV particles and methods to decrease the interaction with, or escape altogether, these neutralizing antibodies would be useful at least for the reason that a lower concentration of rAAV particles that have the ability to evade or escape neutralizing antibodies would be required to be administered to a subject in need of gene therapy compared to rAAV particles without such an ability.

In one aspect, the present disclosure refers to an adeno-associated virus (AAV) capsid protein, wherein the capsid protein comprises a substitution resulting in the amino acid sequence: PGTFNGGKLG (xx1; **SEQ ID NO:191**), RSTFNGDKLN (hi-C; **SEQ ID NO:193**), or PTTFNVDKLG (hi-A; **SEQ ID NO:194**), at the amino acids corresponding to amino acid positions 661 to 670 of the native AAV8 capsid protein (VP1 numbering) based on the reference amino acid sequence of AAV8 identified as **SEQ ID NO:8**. In one example, there is disclosed an adeno-associated virus (AAV) capsid protein, wherein the capsid protein comprises a substitution resulting in the amino acid sequence: X^1-X^2-T-F-N-X^3-X^4-K-L-X^5 (**SEQ ID NO:197**) at the amino acids corresponding to amino acid positions 661 to 670 of the native AAV8 capsid protein (VP1 numbering) based on the reference amino acid sequence of AAV8 identified as **SEQ ID NO:8** and corresponding to amino acid positions 659 to 668 of the native AAV9 capsid protein (VP1 numbering) based on the reference amino acid sequence of AAV9 identified as **SEQ ID NO:26** or at the equivalent amino acid positions in the capsid protein of any other AAV serotype, wherein X^1 is any amino acid other than P; wherein X^2 is any amino acid other than T; wherein X^3 is any amino acid other than Q; wherein X^4 is any amino acid other than S; and/or wherein X^5 is any amino acid other than N.

As an additional example, there is disclosed an adeno-associated virus (AAV) capsid protein, wherein the capsid protein comprises a substitution resulting in the

amino acid sequence: VGTFNEAKLH (h1; **SEQ ID NO:166**), VSTFNPAKLM (h9; **SEQ ID NO:167**), PLTFNCCKLN (d5; **SEQ ID NO:168**), PVTFNQDKLW (d4; **SEQ ID NO:169**), PVTFNSGKLC (h6; **SEQ ID NO:170**), IGTFNGQKLC (h4; **SEQ ID NO:171**), QVTFNGGKLF (h5; **SEQ ID NO:172**), PGTFNGGKLW (h3; **SEQ ID NO:173**), PGTFNGGKLA (h8; **SEQ ID NO:174**), PGTFNRGKLQ (h7; **SEQ ID NO:175**), PGTFNDGKLG (d6; **SEQ ID NO:176**), PSTFNCMKLP (h2; **SEQ ID NO:177**), PSTFNCPKLQ (h11; **SEQ ID NO:178**), PSTFNLGKLS (d1; **SEQ ID NO:179**), PSTFNGGKLP (d7; **SEQ ID NO:180**), SCTFNLHKLC (h12; **SEQ ID NO:181**), QDTFNRTKLC (h10; **SEQ ID NO:182**), PTTFNRTKLM (d3; **SEQ ID NO:183**), FVTFNGDKLM (xx4; **SEQ ID NO:184**), RRTFNSRKLK (xx2; **SEQ ID NO:185**), SVTFNSAKLQ (e2; **SEQ ID NO:186**), VLTFNGSKLA (e1; **SEQ ID NO:187**), PTTFNPSKLW (xx3; **SEQ ID NO:188**), PVTFNEGKLF (e3; **SEQ ID NO:189**), PTTFNQGKLQ (e5; **SEQ ID NO:190**), PGTFNGGKLG (xx1; **SEQ ID NO:191**), PLTFNNGKLS (xx5; **SEQ ID NO:192**), RSTFNGDKLN (hi-C; **SEQ ID NO:193**), PTTFNVDKLG (hi-A; **SEQ ID NO:194**), PITFNEPKLA (hi-B; **SEQ ID NO:195**), or WPTFNAGKLR (hi-e; **SEQ ID NO:196**) at the amino acids corresponding to amino acid positions 661 to 670 of the native AAV8 capsid protein (VP1 numbering) based on the reference amino acid sequence of AAV8 identified as **SEQ ID NO:8** and corresponding to amino acid positions 659 to 668 of the native AAV9 capsid protein (VP1 numbering) based on the reference amino acid sequence of AAV9 identified as **SEQ ID NO:26** or at the equivalent amino acid positions in the capsid protein of any other AAV serotype.

As an additional example, there is described an AAV capsid protein, wherein the capsid protein comprises a substitution resulting in the amino acid sequence: X^1-X^2-T-F-N-X^3-X^4 (**SEQ ID NO:198**) at the amino acids corresponding to amino acid positions 661 to 667 of the native AAV8 capsid protein (VP1 numbering) based on the reference amino acid sequence of AAV8 identified as **SEQ ID NO:8** and corresponding to amino acid positions 659 to 665 of the native AAV9 capsid protein (VP1 numbering) based on the reference amino acid sequence of AAV9 identified as **SEQ ID NO:26** or at the equivalent amino acid positions in the capsid protein of any other AAV serotype, wherein X^1 is any amino acid other than P; wherein X^2 is any amino acid other than T; wherein X^3 is any amino acid other than Q; and/or wherein X^4 is any amino acid other than S.

In addition, the present disclosure is based, at least in part, on the generation of antigenic footprints, or maps, of neutralizing antibodies on AAV particles, and the

development of rAAV particles with amino acid substitutions within these footprints that result in a decreased reactivity to one or more neutralizing antibodies. In particular, the inventors of this disclosure developed antigenic footprints in the 5-fold region of AAV particles.

Accordingly, described herein is a recombinant adeno-associated virus (rAAV) capsid protein comprising a modified 5-fold region, wherein the modified 5-fold region comprises one or more amino acid substitutions. In some examples, one or more amino acid substitutions in a modified 5-fold region results in decreased reactivity to a neutralizing antibody compared to an AAV particle that comprises capsid protein (*i.e.,* VP) without a modified 5-fold region.

In some examples, a rAAV is of serotype 1, and the one or more amino acid substitutions are at positions 250-258, 324-333, 371, 372, 546-550, 557, 655-674 or 716-721 (VP1 numbering) based on the reference amino acid sequence of AAV1 identified as **SEQ ID NO:18**. In some examples, a rAAV is of serotype 2, and the one or more amino acid substitutions are at positions 250-258, 323-332, 370, 371, 545-548, 556, 655-673 or 715-720 (VP1 numbering) based on the reference amino acid sequence of AAV2 identified as **SEQ ID NO:19**. In some examples, a rAAV is of serotype 5, and the one or more amino acid substitutions are at positions 240-248, 314-323, 372, 373, 532-535, 546, 644-662 or 704-709 (VP1 numbering) based on the reference amino acid sequence of AAV5 identified as **SEQ ID NO:22**. In some exampleswhere a rAAV is of serotype 2, and the one or more amino acid substitutions are at positions 250-258, 323-332, 370, 371, 545-548, 556, 655-673 or 715-720, a neutralizing antibody is AVB. In some exampleswhere a rAAV is of serotype 5, and the one or more amino acid substitutions are at positions 240-248, 314-323, 372, 373, 532-535, 546, 644-662 or 704-709, a neutralizing antibody is AVB.

In some examples, a rAAV is of serotype 9, and the one or more amino acid substitutions are at positions 251-255, 264, 325-334 or 656-674 (VP1 numbering) based on the reference amino acid sequence of AAV9 identified as **SEQ ID NO:26**. In some exampleswhere a rAAV is of serotype 9, and the one or more amino acid substitutions are at positions 251-255, 264, 325-334 or 656-674, a neutralizing antibody is CSAL9.

In some examples, a rAAV is of serotype 8, and the one or more amino acid substitutions are at positions 252-266, 326-335, 658-676 or 720 (VP1 numbering) based on the reference amino acid sequence of AAV8 identified as **SEQ ID NO:25**. In some embodiments where rAAV is of serotype 8, and the one or more amino acid substitutions are at positions 252-266, 326-335, 658-676 or 720, a neutralizing antibody is HL2372. In some

examples, a rAAV is of serotype 9, and the one or more amino acid substitutions are at positions 251-266,325-334, 657-673 or 718 (VP1 numbering) based on the reference amino acid sequence of AAV8 identified as **SEQ ID NO:26**. In some exampleswhere rAAV is of serotype 9, and the one or more amino acid substitutions are at positions 251-266,325-334, 657-673 or 718, a neutralizing antibody is HL2372.

In some examples, a rAAV is of serotype 5, and the one or more amino acid substitutions are at positions 218, 240-258, 261, 263, 267, 279, 331, 350, 355-360, 364, 365, 377, 378, 395, 429-432, 437, 450, 451, 453-456, 458, 459, 530-543, 545-548, 639, 641, 642, 648-651, 653-658, 660-662, 697-700 or 704-71 (VP1 numbering) based on the reference amino acid sequence of AAV8 identified as **SEQ ID NO:22**. In some exampleswhere a rAAV is of serotype 5, and the one or more amino acid substitutions are at positions 218, 240-258, 261, 263, 267, 279, 331, 350, 355-360, 364, 365, 377, 378, 395, 429-432, 437, 450, 451, 453-456, 458, 459, 530-543, 545-548, 639, 641, 642, 648-651, 653-658, 660-662, 697-700 or 704-71, a neutralizing antibody is ADK5a.

In some examples, a rAAV is of serotype 5, and the one or more amino acid substitutions are at positions 241-248, 313-319, 321, 323, 355, 356, 358-362, 440-443, 446-449, 530-548, 645-651, 653-661, 697, 698 or 704-712 (VP1 numbering) based on the reference amino acid sequence of AAV8 identified as **SEQ ID NO:22**. In some exampleswhere rAAV is of serotype 5, and the one or more amino acid substitutions are at positions 241-248, 313-319, 321, 323, 355, 356, 358-362, 440-443, 446-449, 530-548, 645-651, 653-661, 697, 698 or 704-712, a neutralizing antibody ADK5b.

In another aspect, the present disclosure also refers to an AAV capsid comprising the AAV capsid protein of this disclosure. In yet another aspect, the present disclosure refers to an AAV virus particle comprising the AAV capsid disclosed herein. In a further aspect, the present disclosure refers to a virus vector comprising: (a) the AAV capsid of claim 3; and (b) a nucleic acid comprising at least one terminal repeat sequence, wherein the nucleic acid is encapsidated by the AAV capsid. Further described herein is a viral vector comprising the AAV capsid of this disclosure as well as a composition comprising the AAV capsid protein, AAV capsid and/or viral vector of this disclosure in a pharmaceutically acceptable carrier. In another aspect, the present disclosure refers to a composition comprising the AAV capsid protein disclosed herein, the AAV capsid disclosed herein, the AAV virus particle disclosed herein, and/or the virus vector disclosed herein, in a pharmaceutically acceptable carrier.

The present disclosure additionally describes a method of introducing a nucleic acid into a cell in the presence of antibodies against the AAV capsid, comprising contacting the cell with the viral vector of this disclosure. In another aspect, the present disclosure refers to a method of administering a nucleic acid to a cell, the method comprising contacting the cell with the virus vector disclosedherein or the composition disclosed herein. The cell can be in a subject and in some embodiments, the subject can be a human subject.

These and other aspects of the invention are addressed in more detail in the description of the invention set forth below.

That which is claimed is:

1. An adeno-associated virus (AAV) capsid protein, wherein the capsid protein comprises a substitution resulting in the amino acid sequence:
PGTFNGGKLG (xx1; **SEQ ID NO:191**), RSTFNGDKLN (hi-C; **SEQ ID NO:193**), or PTTFNVDKLG (hi-A; **SEQ ID NO:194**), at the amino acids corresponding to amino acid positions 661 to 670 of the native AAV8 capsid protein (VP1 numbering) based on the reference amino acid sequence of AAV8 identified as **SEQ ID NO:8**.

2. The AAV capsid protein of claim 1, wherein the substitution is RSTFNGDKLN (hi-C; **SEQ ID NO:193**).

3. An AAV capsid comprising the AAV capsid protein of claim 1 or claim 2.

4. An AAV virus particle comprising the AAV capsid of claim 3.

5. A virus vector comprising:
(a) the AAV capsid of claim 3; and
(b) a nucleic acid comprising at least one terminal repeat sequence, wherein the nucleic acid is encapsidated by the AAV capsid.

6. The virus vector of claim 5, wherein the nucleic acid comprises a sequence encoding a therapeutic protein or therapeutic RNA.

7. The virus vector of claim 5, wherein the virus vector is immunologically distinct from its parental AAV serotype and is not recognized by antibodies that bind the parental serotype.

8. A composition comprising the AAV capsid protein of any of claims 1 or 2, the AAV capsid of claim 3, the AAV virus particle of claim 4, and/or the virus vector of any one of claims 5 to 7, in a pharmaceutically acceptable carrier.

9. A method of administering a nucleic acid to a cell, the method comprising contacting the cell with the virus vector of any one of claims 5 to 7 or the composition of claim 8.