

the central nervous systems (CNS) and peripheral nervous systems (PNS). The blood brain barrier can impede access of the AAV-based therapy to the CNS when administered systemically and direct administration to CNS tissues can involve invasive surgeries. Further, the high doses of AAV-based therapies necessary to yield sufficient transduction of target CNS and PNS tissues increases the risk of side effects, and/or undesired immune responses. Additionally, the need to produce high doses of AAV poses a manufacturing burden.

[006] Known AAV serotypes each have a specific tissue tropism, and there are some tissues (e.g., kidney) that cannot be easily targeted using these AAVs. Additionally, AAV transduction in systemic organs such as the heart, liver or lung can vary significantly for a given dose in various model organisms used during clinical development (e.g., canine, pig, non-human primates) and in human subjects. This inability to accurately test AAV-based therapies in animal models prior to human use is also problematic.

[007] As the scope of AAV gene transfer applications has expanded, including the advancement of gene-therapies to CNS and/or PNS disorders, there remains a need in the art to address differences in AAV tropism across different species. These differences often result in non-linear vector dose biodistribution relationships when scaling from small to large animal models – subsequently impacting clinical translation. As such, there is an unmet need in the art to develop AAV gene delivery platforms with greater translatability across multiple species. Additionally, there is a need to develop AAV-based gene therapies that are able to selectively and specifically target tissues of interest, including tissues that are have been difficult to target using known AAV serotypes.

BRIEF SUMMARY OF THE DISCLOSURE

[008] The present disclosure provides, at least in part, methods and compositions comprising an adeno-associated virus (AAV) capsid protein, comprising one or more amino acid substitutions, wherein the substitutions introduce into an AAV vector comprising these modified capsid proteins one or more improved functionalities such as, but not limited to, the ability to evade host antibodies, selective tropism, and/or higher transduction efficiency.

[009] An aspectexample of the present disclosure provides for recombinant AAV vectors comprising an AAV capsid protein variant as disclosed herein. In some embodimentexamples, recombinant AAV vectors herein may comprise an AAV capsid protein variant, wherein the capsid protein variant comprises a peptide having the sequence of any one of SEQ ID NOs: 2-19. In some embodimentexamples, recombinant AAV vectors herein comprises an AAV capsid

protein variant, wherein the capsid protein variant has at least 90% identity to the sequence of SEQ ID NO: 1, wherein the amino acids corresponding to amino acids 452-458 of SEQ ID NO: 1 are substituted with a peptide having a sequence of any one of SEQ ID NOs: 20-28. In some [embodimentexamples](#), recombinant AAV vectors herein may comprise an AAV capsid protein variant, wherein the capsid protein variant has at least 90% identity to the sequence of SEQ ID NO: 1, wherein the amino acids corresponding to amino acids 586-592 of SEQ ID NO: 1 are substituted with a peptide having a sequence of any one of SEQ ID NOs: 29-37.

[009a] In one aspect, the present disclosure refers to an AAV vector comprising an AAV capsid protein comprising the sequence of any one of SEQ ID NOs: 2-19.

[0010] In some [embodimentexamples](#), recombinant AAV vectors herein comprises an AAV capsid protein variant, wherein the capsid protein variant has at least 90% identity to the sequence of SEQ ID NO: 1, wherein the amino acids corresponding to amino acids 452-458 of SEQ ID NO: 1 are substituted with a peptide having a sequence of any one of SEQ ID NOs: 20-28; and wherein the amino acids corresponding to amino acids 586-592 of SEQ ID NO: 1 are substituted with a peptide having a sequence of any one of SEQ ID NOs: 29-37.

[0011] In some [embodimentexamples](#), recombinant AAV vectors herein may comprise an AAV capsid protein variant, wherein the capsid protein variant has the sequence of any one of SEQ ID NO: 2-19, 46-123 or a sequence with at least 90% or at least 95% identity thereto. In some [embodimentexamples](#), recombinant AAV vectors herein comprise an AAV capsid protein variant, wherein the capsid protein variant has the sequence of any one of SEQ ID NO: 2-19, 46-123 or a sequence with 1-10, 11-20, 20-30, or 30-50 amino acid substitutions relative thereto.

[0012] Another [aspectexample](#) of the present disclosure provides for AAV capsid protein variants as disclosed herein. In some [embodimentexamples](#), AAV capsid protein variants herein comprise a peptide having the sequence of any one of SEQ ID NOs: 2-19. In one aspect, the present disclosure refers to an AAV capsid protein comprising the sequence of any one of SEQ ID NOs: 2-19.

[0013] In some [embodimentexamples](#), AAV capsid protein variants herein have at least 90% identity to the sequence of SEQ ID NO: 1, wherein the amino acids corresponding to amino acids 452-458 of SEQ ID NO: 1 are substituted with a peptide having a sequence of any one of SEQ ID NOs: 20-28. In some [embodimentexamples](#), AAV capsid protein variants herein have at least 90% identity to the sequence of SEQ ID NO: 1, wherein the amino acids corresponding to amino acids 586-592 of SEQ ID NO: 1 are substituted with a peptide having a sequence of any one of SEQ ID NOs: 29-37.

[0014] In some [embodimentexamples](#), AAV capsid protein variants herein may have at least 90% identity to the sequence of SEQ ID NO: 1, wherein the amino acids corresponding to amino acids 452-458 of SEQ ID NO: 1 may be substituted with a peptide having a sequence of any one of SEQ ID NOs: 20-28; and wherein the amino acids corresponding to amino acids 586-

592 of SEQ ID NO: 1 may be substituted with a peptide having a sequence of any one of SEQ ID NOs: 29-37.

[0015] In some embodimentexamples, AAV capsid protein variants herein may have the sequence of any one of SEQ ID NO: 2-19, 46-123 or a sequence with at least 90% or at least 95% identity thereto. In some embodimentexamples, capsid protein variants herein may have the sequence of any one of SEQ ID NO: 2-19, 46-123 or a sequence with 1-10, 11-20, 20-30, or 30-50 amino acid substitutions relative thereto.

[0016] Another aspectexample of the present disclosure provides for pharmaceutical compositions comprising any of the of the AAV capsid protein variants and/or AAV vectors disclosed herein. In some embodimentexamples, pharmaceutical compositions herein may further comprise at least one pharmaceutically acceptable carrier.

[0017] Another aspectexample of the present disclosure provides for methods of introducing a recombinant AAV vector into a target cell. In one aspect, the present disclosure refers to a method of introducing a transgene into a cell, the method comprising: contacting the cell with the AAV vector as disclosed herein. In some embodimentexamples, the methods of introducing a recombinant AAV vector into a target cell herein may include contacting the target cell with any of the recombinant AAV vectors (e.g., ccAAVs) and/or pharmaceutical compositions disclosed herein. In some embodimentexamples, methods herein can deliver one or more therapeutic heterologous molecules to a target cell in a subject, the methods comprising administering to the subject any of the recombinant AAV vectors (e.g., ccAAVs) and/or pharmaceutical compositions disclosed herein. In some embodimentexamples, any of the any of the recombinant AAV vectors (e.g., ccAAVs) and/or pharmaceutical compositions disclosed herein can be administered to a subject by intramuscular injection, intravenous injection, intracoronary injection, intraarterial injection, or any combination thereof. In one aspect, the present disclosure refers to use of the AAV vector as disclosed herein in the manufacture of a medicament for delivering a transgene to a cell in a subject.

[0018] Another aspectexample of the present disclosure provides for methods of evolving novel strains of adeno-associated viruses comprising passaging AAV capsid libraries across multiple mammalian species. In some embodimentexamples, methods herein may utilize AAV capsid libraries comprising AAV capsids packaging different genomes encoding mutagenized capsid gene sequences. In some embodimentexamples, methods herein may administer AAV capsid libraries herein to *Mus Musculus* (mouse), *Sus scrofa* (pig), *Canis Familiaris* (Dog), Non-human primates (*Macaca*, macaque), or *Homo sapiens* (human), and any combination or repeated cycles thereof. In some embodimentexamples, methods herein may enrich adeno-associated virus (AAV) capsid protein sequences herein by passaging the AAV capsid libraries according to the methods disclosed herein. In some embodimentexamples, methods herein may enrich sequences encoding capsid protein variants herein by extracting the AAV capsid protein variants from cells collected or derived from the group consisting of spinal cord (e.g., glial cells, neurons,

endothelial cells), dorsal root ganglion, brain, heart, lung, kidney, skeletal muscle, spleen, pancreas, small intestine, large intestine, or liver tissue, and any combination thereof. In some embodimentexamples, methods herein may produce AAV capsid protein variants as disclosed herein with improved gene transfer efficiency in any mammalian species selected from the group consisting of: *Mus Musculus* (mouse), *Sus scrofa* (pig), *Canis Familiaris* (Dog), Non-human primates (*Macaca*, macaque), or *Homo sapiens* (human), and any combination or repeated cycles thereof. In some embodimentexamples, methods herein may produce AAV capsid protein variants as disclosed herein with improved gene transfer efficiency in any of the cell types or tissues the group consisting of spinal cord, dorsal root ganglion, brain, heart, lung, kidney, skeletal muscle, spleen, pancreas, small intestine, large intestine, or liver tissue, and any combination thereof. In some embodimentexamples, methods herein may produce AAV capsid protein variants as disclosed herein with improved immune response in any of the cell types or tissues the group consisting of spinal cord, dorsal root ganglion, brain, heart, lung, kidney, skeletal muscle, spleen, pancreas, small intestine, large intestine, or liver tissue, and any combination thereof. In some embodimentexamples, methods herein may produce AAV capsid protein variants as disclosed herein with improved tropism in any of the cell types or tissues the group consisting of spinal cord, dorsal root ganglion, brain, heart, lung, kidney, skeletal muscle, spleen, pancreas, small intestine, large intestine, or liver tissue, and any combination thereof.

[0018a] In one aspect, the present disclosure refers to a plasmid comprising an AAV cap gene encoding the AAV capsid protein as disclosed herein.

[0018b] In one aspect, the present disclosure refers to a cell line that stably expresses the AAV capsid protein as disclosed herein.

[0019] An aspectexample of the disclosure provides for kits, wherein a kit can comprise any of the compositions or AAV vectors disclosed herein and at least one container.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, which can be better understood by reference to the drawings in combination with the detailed description of specific embodiments presented herein.

[0021] **Figs. 1A and 1B** illustrate bubble plots showing analysis of library diversity, directed evolution and enrichment of AAVs comprising capsid proteins with novel peptide substitutions in accordance with certain embodiments herein. Parental (*Fig. 1A*) and evolved libraries from three cycles (*Fig. 1B*) were subjected to high-throughput sequencing using the Illumina MiSeq platform. Following analysis with a custom Perl script, enriched amino acid sequences were plotted. Each bubble represents a distinct capsid protein amino acid sequence with the radius of the bubble proportional to the number of reads for that variant in the