Characterization of Receptor-Targeted Blood-Brain Barrier Penetrant AAV Capsids

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Poster #1896

Introduction

- Blood-brain barrier (BBB) penetrant adeno-associated virus (AAV) capsids have the potential to transform the treatment of neurological disorders by enabling efficient intravenous delivery of genomic medicines to the central nervous system (CNS).
- AAV capsids can be engineered to interact with receptors that mediate transcytosis of the blood-brain barrier. Here, we report the characterization of AAV capsid families that are engineered to target transferrin receptor (TFRC), tissue-nonspecific alkaline phosphatase (ALPL), or low-density lipoprotein receptor (LDLR). A subset of capsids in each family exhibit cross-species receptor interaction in humans and nonhuman primates.
- These receptor-targeted capsid families are unrelated to our STAC-BBB family (see abstract #1909) but likewise have the potential to mediate widespread CNS delivery.

Receptor-targeted capsid engineering

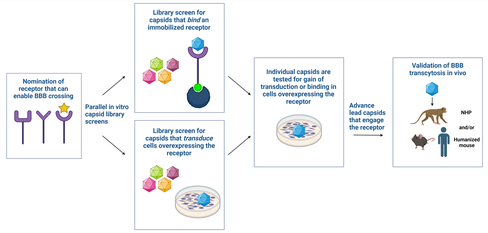

Figure 1. Receptor-targeted capsid engineering approach.
Capsid libraries are screened in parallel for binding to the immobilized receptor and for transduction of cells that overexpress the receptor. Capsids that specifically target the receptor are evaluated individually to confirm targeting specificity and assess cross-species performance. Lead capsids are advanced for in vivo assessment of BBB transcytosis and CNS delivery.

Species cross-reactive capsids targeting TFRC

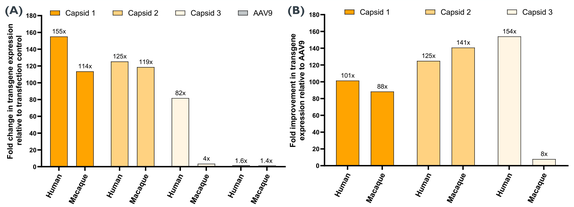

Figure 2. Engineered capsids exhibit enhanced transduction of cells expressing transferrin receptor.
Neuro2A cells were transfected with a plasmid encoding human TFRC, cynomolgus macaque TFRC, or a transfection control. Transfected cells were transduced with lead AAV capsids and transgene expression was assessed 72 hours later by RT-qPCR.
(A) Fold increase in transgene expression relative to transfection control. Capsids 1 and 2 exhibit enhanced transgene expression in cells overexpressing human or macaque TFRC. In contrast, capsid 3 exhibits significant enhancement of transduction only in cells expressing human TFRC. AAV9 exhibits no enhancement of transduction in cells expressing TFRC.
(B) Engineered capsids mediate higher transgene expression than AAV9 in cells overexpressing the human or macaque orthologs of TFRC.

Binding kinetics of capsids targeting TFRC

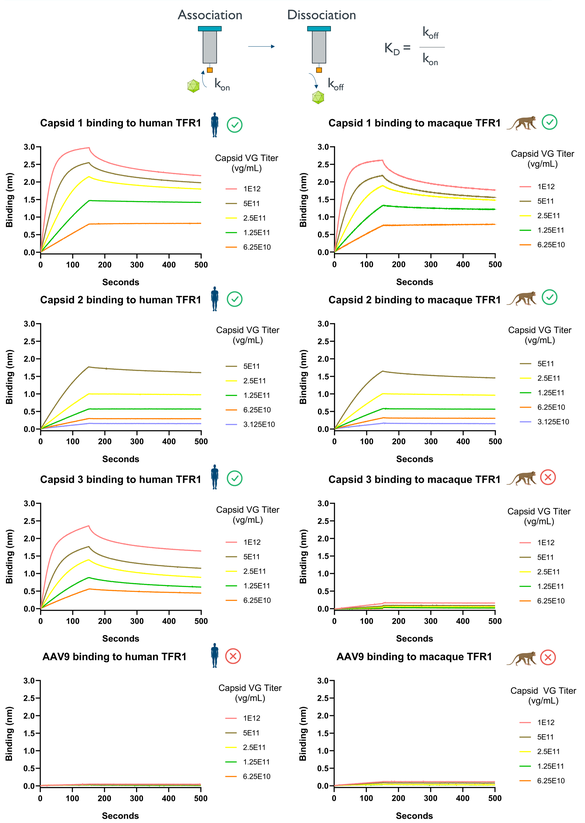

Figure 3. Binding kinetics of engineered capsids targeting transferrin receptor.
Human or macaque transferrin receptor was immobilized on Octet BLI sensors and binding to each capsid analyte was measured across a range of capsid concentrations. Three lead capsids demonstrated robust binding responses to human transferrin receptor, while the negative control AAV9 did not bind. Importantly, capsids 1 and 2 exhibit similar binding responses for both human and macaque transferrin receptor. Capsid 3 binds to human transferrin receptor, but not macaque. Dissociation constants for capsids 1-3 were determined to be in a low pM range. These results are concordant with cross-species performance observed in cell culture experiments.

Species cross-reactive capsids targeting ALPL

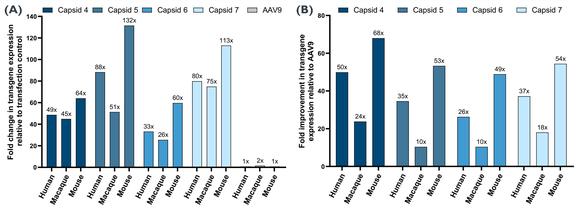

Figure 4. Engineered capsids exhibit enhanced transduction of cells expressing the human, macaque, or mouse orthologs of ALPL.
HEK293 cells were transiently transfected with a plasmid encoding human, cynomolgus macaque, or mouse ALPL. Transfected cells were transduced with lead AAV capsids at an MOI of 3000 and transgene expression was assessed 72 hours later by RT-qPCR.
(A) Fold increase in transgene expression relative to a transfection control. Capsids 4-7 exhibit enhanced transgene expression in cells overexpressing human, macaque, or mouse ALPL, but not in the transfection control condition.
(B) Engineered capsids mediate higher transgene expression than AAV9 in cells overexpressing human, macaque, or mouse ALPL.

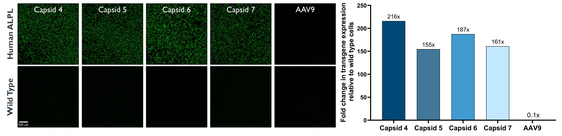

Figure 5. Enhanced transduction in a stable cell line expressing human ALPL.
A lentiviral vector was used to generate a stable cell line expressing human ALPL. Cells were transduced at an MOI of 3E5 with AAV vectors encoding a nuclear localized GFP. Native GFP fluorescence and transgene expression levels were assessed 72 hours post-transduction. Capsids 4-7 mediate enhanced GFP expression in cells expressing human ALPL compared to wild type cells.

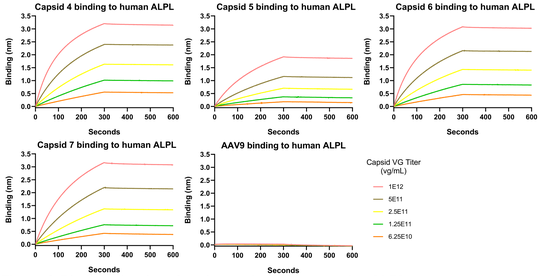

Figure 6. Binding kinetics of engineered capsids targeting ALPL.
Human ALPL receptor was immobilized on Octet BLI sensors and binding to each capsid analyte was measured across a range of capsid concentrations. Four lead capsids demonstrated robust binding responses to human ALPL, while the negative control AAV9 did not bind. Dissociation constants for capsids 4-7 were determined to be in a low pM range.

Species cross-reactive capsids targeting LDLR

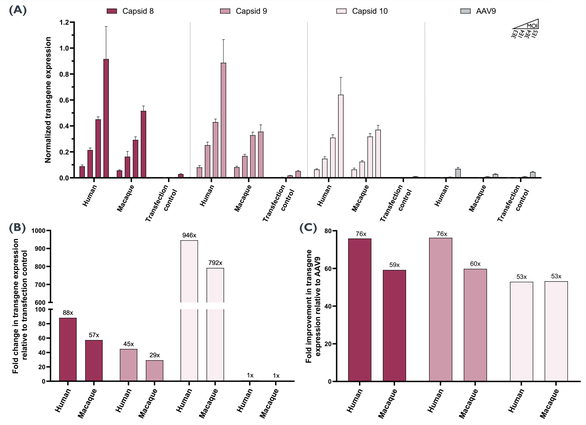

Figure 7. Engineered capsids exhibit enhanced transduction of cells expressing the human and macaque orthologs of LDLR.
Neuro2A cells were transiently transfected with a plasmid encoding human LDLR, cynomolgus macaque LDLR, or a transfection control. Transfected cells were transduced with each AAV and transgene expression was assessed 72 hours later by RT-qPCR.
(A) Capsids 8-10 were evaluated individually and compared against the parental serotype AAV9. The engineered capsids exhibit enhanced transgene expression in cells overexpressing human or macaque LDLR, but not in the transfection control condition.
(B) Fold increase in transgene expression relative to a transfection control. A nonlinear regression model was used to interpolate relative transgene expression values for each capsid-receptor condition. These values were then scaled to the transfection control value for each capsid.
(C) Engineered capsids mediate higher transgene expression than AAV9 in cells overexpressing the human or macaque orthologs of LDLR.

Conclusions and next steps

- We have engineered receptor-targeted AAV capsids that engage transferrin receptor (TFRC), tissue-nonspecific alkaline phosphatase (ALPL), or low-density lipoprotein receptor (LDLR).
- A subset of engineered capsids targeting transferrin receptor exhibits cross-species binding to both the human and macaque orthologs, along with enhanced transduction in cells that express TFRC. To our knowledge, this represents the first example of an engineered capsid that binds both human and macaque transferrin receptors without requiring fusion to an orthogonal targeting ligand.
- A second family of capsids targeting ALPL exhibits enhanced transduction in cells expressing the human, macaque, or mouse receptor orthologs. Lead capsids also demonstrate robust binding responses to human ALPL.
- A third family of capsids targeting LDLR exhibits cross-species enhancement of transduction in cells expressing the human or macaque receptor orthologs.
- In the progression from preclinical to clinical testing, cross-reactive receptor binding in humans and nonhuman primates is a critical translational consideration. These receptor-targeted capsids will be evaluated in vivo to assess BBB transcytosis and tropism for CNS cell types.

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