Fitness maturation of STAC-BBB yields second-generation capsid variants with enhanced delivery to the central nervous system

Matthew Tiffany, Stephanye Frias, Lori Andrews, Ankitha Nanjaraj, Esmeralda Ponce, Satria Sajuthi, Russell Darst, Yuri Bendaña, Gillian Houlihan, Matthew Mendel, Hung Tran, Sarah Mueller, Bryan Zeitler, David Ojala
All authors are current or former employees of Sangamo Therapeutics, Inc., Richmond, CA.

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Introduction

- STAC-BBB is an engineered AAV capsid that mediates widespread central nervous system (CNS) transduction in cynomolgus macaques after intravenous administration.
- We have identified a putative receptor for STAC-BBB and demonstrated that overexpression of the human, macaque, or mouse orthologs of this receptor confer enhanced transduction for STAC-BBB in vitro.
- Here we report that STAC-BBB also crosses the blood-brain barrier in mice and mediates widespread neuronal transduction throughout the CNS. This result suggests that STAC-BBB crosses the blood-brain barrier using a mechanism that is highly conserved across species.
- We additionally conducted fitness maturation of STAC-BBB to identify second-generation STAC-BBB variants that exhibit improved CNS delivery in both nonhuman primates and mice. These library studies demonstrate a strong correlation between STAC-BBB variant performance across species.
- Finally, we utilized biolayer interferometry to show that both STAC-BBB and second-generation STAC-BBB variants bind to the putative receptor with high affinity.
- Taken together, these results support the advancement of STAC-BBB and second-generation STAC-BBB variants towards clinical studies for the treatment of neurological disorders.

STAC-BBB mediates widespread brain delivery in both nonhuman primates and mice

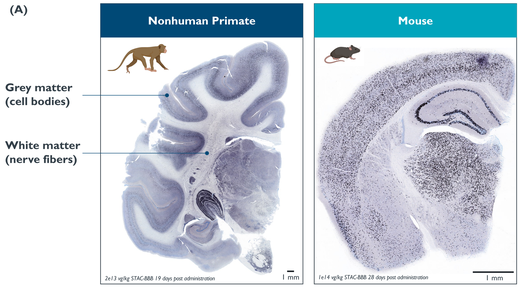
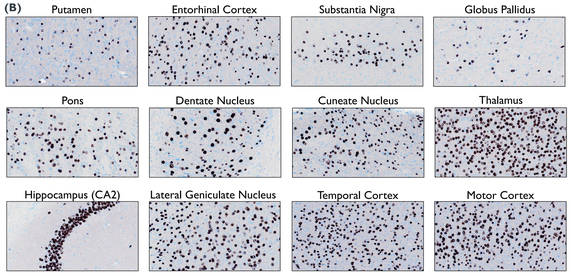


Figure 1. STAC-BBB delivery of nuclear localized GFP in NHP and mouse.
(A) Immunohistochemical staining for nuclear localized GFP expression in macaque and mouse brain shows high levels of expression throughout the grey matter, indicating widespread CNS delivery in both species. Antibody labeling of nuclear localized GFP is shown in black. A nissl stain in light blue detects all cell nuclei.
(B) Representative images of STAC-BBB mediated GFP expression in mouse brain regions that are associated with a variety of neurological disorders.



Design of STAC-BBB fitness maturation libraries

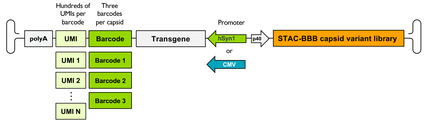


Figure 3. Library design for STAC-BBB fitness maturation libraries. Mutations were introduced in the STAC-BBB capsid gene and each variant was linked to distinct barcodes using a bioinformatic look-up table. Hundreds of unique molecular identifiers (UMIs) were cloned per barcode. The barcoded mRNA transcript is expressed from a neuron specific hSynapsin1 promoter or a ubiquitous CMV promoter, enabling multiplexed assessment of capsid tropism.

Performance of STAC-BBB fitness maturation library is highly correlated between NHP and mouse

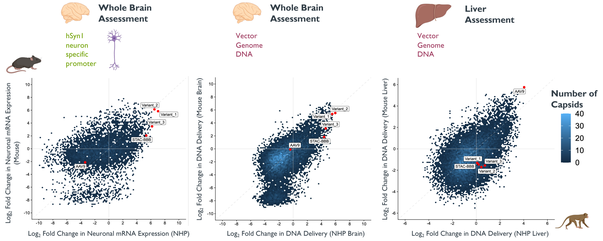


Figure 4. Correlation of STAC-BBB fitness maturation library performance in NHP and mouse. The STAC-BBB fitness maturation library was administered intravenously in adult macaques and mice. The density correlation plots show the $\log_2$ fold change in capsid delivery between NHP (x-axis) and mouse (y-axis). The color indicates the local density of variants, with lighter colors representing areas with higher capsid counts. AAV9, STAC-BBB, and second-generation STAC-BBB variants 1-4 are annotated. A COV threshold was applied to remove high variance capsids. The results support a strong correlation between the performance of STAC-BBB variants in macaque and mouse.

Second-generation STAC-BBB variants exhibit enhanced CNS transduction in NHP and mouse

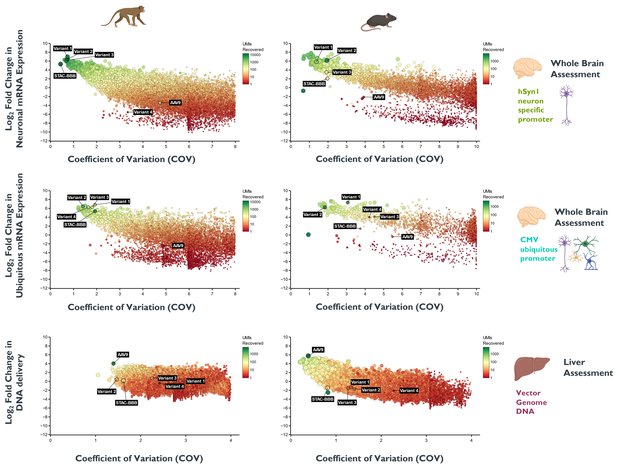


Figure 5. Evaluation of STAC-BBB fitness maturation library in NHP and mouse. The left and right columns of bubble plots show results from cynomolgus macaque and mouse, respectively. The Y-axis represents the $\log_2$ fold change in delivery for each capsid variant relative to its input abundance in the library. The X-axis represents the coefficient of variation (COV). The color represents the number of unique molecular identifiers (UMIs) detected for a capsid variant, and the bubble size corresponds to the fraction of replicates in which the capsid variant is found. Top performing capsids for brain delivery are found in the upper left of the bubble plots. Second-generation STAC-BBB variants 1 through 4 are annotated along with the controls STAC-BBB and AAV9. **Top row**: performance of library in whole brain neuronal mRNA expression. **Middle row**: performance of the library in whole brain ubiquitous mRNA expression, highlighting Variant 4, a capsid variant that exhibits an apparent shift towards transduction of a non-neuronal cell type. **Bottom row**: DNA delivery to the liver.

	Macaque			Mouse			In vitro		
Capsid	Whole Brain Neuronal RNA	Whole Brain Ubiquitous RNA	Liver DNA	Whole Brain Neuronal RNA	Whole Brain Ubiquitous RNA	Liver DNA	Human Receptor	Macaque Receptor	Mouse Receptor
STAC-BBB	1	1	1	1	1	1	✓	✓	✓
Variant 1	3.2	2.8	0.9	13.8	59.6	1.1	✓	✓	✓
Variant 2	2.2	2.1	1.2	16.9	28.4	0.9	✓	✓	✓
Variant 3	1.8	1.9	1.5	2.7	6.5	0.9	✓	✓	✓
Variant 4	0.001	1.9	0.7	0.002	6.0	0.7	✓	✓	✓

Table 1. STAC-BBB fitness maturation library performance in mouse and NHP. Normalized fold change values relative to STAC-BBB are shown for second-generation variants 1-4. Second-generation STAC-BBB variants exhibit enhanced brain delivery in macaque and mouse. Like STAC-BBB, these second-generation capsids are detargeted from the liver and exhibit cross-species interaction with Receptor 1.

STAC-BBB and second-generation variants directly bind Receptor 1 with high affinity

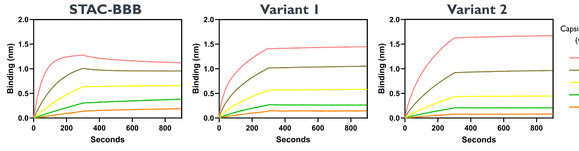


Figure 6. Binding kinetics between Receptor 1 and STAC-BBB plus select second-generation variants. Human Receptor 1 was immobilized on Octet BLI sensors and binding to analytes STAC-BBB or second-generation Variants 1 and 2 was measured across a range of capsid concentrations. All three capsids demonstrated robust binding responses to the human ortholog of Receptor 1. Variants 1 and 2 exhibit slower dissociation rates compared to STAC-BBB, suggestive of enhanced binding. In separate experiments STAC-BBB bound human Receptor 1 with a dissociation constant in the low pM range while AAV9 did not bind.

Overexpression of human, macaque, and mouse Receptor 1 confers enhanced transduction for STAC-BBB and second-generation variants

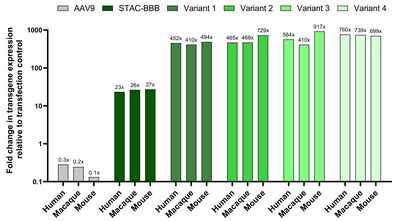


Figure 7. Second-generation STAC-BBB variants exhibit cross-species interaction with Receptor 1 in vitro. HEK293 cells were transiently transfected with plasmids encoding Receptor 1 orthologs from human, macaque, or mouse. A barcoded vector pool comprised of STAC-BBB, second-generation STAC-BBB variants, and AAV9 was used to transduce the cells at an MOI of 1E5. Barcoded transgene expression was assessed 72-hours post transduction by next-generation sequencing. The data represents the fold change in barcoded transgene expression normalized to the input abundance of each capsid.

Conclusions

- STAC-BBB crosses the blood-brain barrier in both macaques and mice.
- Second-generation STAC-BBB variants, identified by fitness maturation library selection, show a 3 to 17-fold improvement in CNS transduction in macaque and mouse, respectively.
- STAC-BBB binds to human Receptor 1 with high affinity. Second-generation variants that mediate enhanced CNS delivery in vivo also exhibit enhanced binding to human Receptor 1.
- Overexpression of human, macaque, and mouse Receptor 1 orthologs in vitro enhances transduction with STAC-BBB and second-generation STAC-BBB variants.
- These second-generation STAC-BBB capsids will be evaluated individually in vivo.
- Taken together, these results support the advancement of STAC-BBB and second-generation STAC-BBB variants towards clinical studies for the treatment of neurological disorders.

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