Structure-Guided Engineering of Ancestrally Derived AAV Capsids: Enhancing Precision Gene Therapy Through the Rational De-Targeting of Hepatic Tropism

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Approximately 5.9% of the global population—over 300 million people—suffer from rare genetic disorders, many of which require AAV-based gene therapy. AAV-B3, an ancestrally derived serotype with promising brain-targeting capabilities, presents a major challenge: heightened hepatic tropism. This leads to off-target liver transduction, hepatotoxicity, immune activation, dose escalation, and inflated treatment costs—barriers that limit the safety and accessibility of gene therapy. To address this, I employed a five-phase engineering pipeline to rationally reduce AAV-B3 liver tropism. N271A and N271T mutations were introduced into Loop 1 of the hypervariable region of the cap gene and integrated into the PHLP-B3 plasmid via PCR, NEB Hi-Fi assembly, electroporation, and plasmid cloning. Recombinant AAV was produced using triple-plasmid transfection. After testing, N271A and N271T showed liver transduction reductions of 88% and 39%, respectively. To scale this approach, I developed TropiMut-3D, a machine learning model trained on 100+ AAV capsid mutations using 16 biochemical, structural, and positional features. The model achieved strong predictive performance with approximately 89% accuracy, enabling cost-effective in-silico screening of mutations prior to wet lab testing. Together, this dual computational–experimental framework demonstrates that rational AAV engineering can dramatically reduce off-target liver transduction—mitigating toxicity, reducing cost, and enhancing precision in gene therapy. Future work will focus on saturation mutagenesis of Loop 1 guided by TropiMut-3D, along with large-animal studies and preclinical validation to advance the safety and scalability of brain-directed AAV vectors.

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