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Abstract

Adeno-Associated Virus (AAV) is a reportedly helper-dependent and non-pathogenic virus. A capsid surrounds the 4.7 kb AAV genome flanked by hairpin-loop inverted terminal repeats (ITRs).[1] Rep proteins bind the Rep-Binding Element (RBE) on the ITRs and nick at the terminal resolution site (trs) through tyrosine (Y156) to replicate DNA.[2] Rep proteins have three replication domains: the DNA-Binding Loop (L_{DB}), the alpha-D (αD) domain, and the αE domain (αE) which contains Y156.[3] Flexible binding allows some Rep serotypes like Rep2 to non-specifically replicate other ITR serotypes.[4] This along with Rep being common in the human body causes non-specific replication which can lead to gene overexpression or spread of therapeutics to non-targeted regions. Creating an ITR-Rep protein complex unable to be replicated by another Rep serotype is necessary for safer AAV gene delivery. To create a unique interaction, protein models were used to guide rational mutagenesis. Rep2 was modeled using RoseTTAFold and PyMOL.[5,6,7] Nicking proposed to create specificity was done by altering the structural position of Y156 by replicating the second turn of the helix while maintaining the helical structure of the αE domain to create a unique nick site.[4,2] This mutant (thB) was hypothesized to allow only for a mutant ITR to be replicated. The production of an ITR replicated by thB will create a uniquely binding complex for safer gene delivery.

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