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Dissecting AAV Structure-Function Correlates Through Directed Evolution

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The three-fold axis of symmetry of parvoviruses contains structures critical for infectivity, including determinants of tropism and transduction efficiency. This region is also commonly targeted by neutralizing antibodies arising from humoral responses to the viral capsid. In case of Adeno-Associated Viruses (AAV), a particularly interesting example is AAV serotype 8, for which the three-fold axis has been shown to be important for both cell attachment and post-entry processing. Here, we utilize structure-guided evolution to understand the role of different variable regions on the AAV8 capsid and demonstrate that different surface footprints can be engineered to impart distinct biological properties. Two novel variants, dubbed AAV840 and AAV880, with engineered variable regions IV and VIII, respectively, show 1-2 orders of magnitude higher transduction than AAV8 vectors in vitro. The more potent AAV840 variant shows increased cell surface binding and cellular uptake, while AAV880 shows improved cell surface binding. Additionally, we have identified distinct cell surface attachment receptors for each mutant. Assays utilizing glycan-deficient cell lines and a panel of lectins revealed that AAV840 utilizes the trimannosyl core of an N-linked glycan, while competitive inhibition assays demonstrated AAV880 exploits sulfated glycosaminoglycans for cell attachment. Moreover, AAV880 is no longer recognized by several monoclonal antibodies targeting the surface spikes on the AAV8 three-fold symmetry axis, demonstrating overlap between antigenic and receptor footprints. Our studies suggest that different surface loops on AAV capsids can be engineered to play distinct roles in host cell surface recognition and entry.