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Characterizing a Novel Antibody Epitope on the AAV5 Capsid at Atomic Resolution for the Creation of Host Antibody Resistant Recombinant Gene Delivery Vectors

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Adeno-associated virus serotype 5 (AAV5) is being developed as a gene delivery vector for several diseases, including cystic fibrosis, hemophilia, and Huntington's disease, and has demonstrated successful transduction of liver, lung, skeletal muscle, and the central nervous system. One of the limitations of the AAVs for gene delivery are pre-existing neutralizing antibodies in the human population. While the seroprevalence of AAV5 is lower than other serotypes, low levels of pre-existing immunity still presents a significant challenge for vector effectiveness in gene delivery applications. Here we report the cryo-electron microscopy and image reconstructed (cryo-EM) structure of AAV5 in complex with the newly generated monoclonal antibody HL2476 to a resolution of 3.1 Å. To our knowledge, this is the highest-resolution antibody complex structure determined by cryo-EM to date. Unlike the other available anti-AAV5 capsid antibodies, ADK5a and ADK5b, with epitopes surrounding the 5-fold channel of the AAV5 capsid, HL2476 binds to the 3-fold protrusions. In addition, to fully understand the capsid-antibody interactions, the heavy and light chains of HL2476 were sequenced and built into the cryo-EM density map along with the AAV5 VP structure. The high resolution of the complex enabled the identification of AAV5 capsid residues, in previously defined variable regions (VR) VR-V, VR-VI, and VR-VIII, involved in the antibody interaction. To confirm this a comprehensive panel of site-directed mutants, guided by the structures of AAV5 complexed with HL2476, was generated. Native dot blot analysis and transduction assays, in the absence / presence of the parental MAb are used to test the variants arising from this study to characterize antibody escape phenotypes. These studies identify single or few footprint residues as determinants of these interactions and highlight hotspots for engineering host immune escape vectors.