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Structural Insights into the Role of the Conserved Leucine Located at the Base of the 5-Fold Pore in AAV Infectivity

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Adeno-associated virus (AAV) is a gene therapy vector. Designing the next generation of AAV vectors requires understanding of the viral life cycle and critically conserved residues. AAV2's L336 is conserved in virtually all *Parvovirinae* at the equivalent position. This residue is located at the narrowest point in the 5-fold channel of the capsid. The 5-fold channel is postulated to be where AAV's VP1 unique N-terminal region (VP1u) containing a PLA_2 is externalized from the capsid for its function. PLA_2 is predicted to be required for escape from the late endosome in viral infection. In previous studies altering this residue led to defect in infection proposed to be due to VP1u either being stuck inside the capsid or prematurely disposed on the outside of the capsid. In an attempt to understand the loss of infectivity, the structure of wild type (WT) AAV2, AAV2-L336C, and AAV2-L336W were determined to atomic resolution (currently at 2.30, 1.86, and 2.70 Å resolution, respectively) by cryo-electron microscopy and image reconstruction to visualize potential conformational change(s) in the capsid which could explain the loss of infectivity in these variants. In addition to local residue rearrangements proximal to position 336 in the variants at the base of the 5-fold channel compared to WT AAV2, the VP N-terminus are less ordered, by ~10 to 20 residues, compared to WT AAV2. The disordered N-terminus of the variant structures not only open up the base of the 5-fold channel, it implies flexibility of the VP1u, VP1/2 overlap, and VP3 N-terminus that could affect the PLA_2 externalization and hence its function. A comparative analysis of these two variants with WT AAV2 will be presented along with the implications of the structure in their defective phenotype.