

Featured Publication Note

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Three-dimensional rendering of adeno-associated viral infection

Adeno-associated virus (AAV) serotypes have many properties which make them attractive vectors for gene therapy. However, before these vectors can be applied as effective tools in gene therapy, researchers need to overcome several barriers to gene delivery, one of which is at the level of subcellular processing of the virion. Previous studies have identified agents that overcome these subcellular barriers and enhance transduction, including proteasome inhibitors and hydroxyurea (HU), although the mechanisms of action of these agents are unclear. In this study, scientists set out to understand how cellular parameters control the processing of virions, by tracking the subcellular fate of recombinant AAV2 (rAAV2) vectors. **Volocity** software aided this work by allowing researchers to perform a 3D infection analysis. This research will hopefully lead to improved gene delivery through exploitation of these cellular parameters and improved vector design.

Researchers used **Volocity** to explore nucleolar accumulation of rAAV2 virions in 3D by rendering z-stacked images acquired by confocal microscopy (figure). Using **Volocity**, they were able to digitally subtract the nucleus by channel gating to reveal more precisely the localization of capsids in its interior. rAAV2 virions were shown to colocalize with the nucleolar marker NCL after infection, whereas empty capsids did not accumulate in nucleoli or colocalize with NCL, even in the presence of a proteasome inhibitor which enhances transduction.

The researchers showed that proteasome inhibitors can potentiate nucleolar accumulation, whilst HU reduces nucleolar accumulation and mobilized capsids to the nucleoplasm, suggesting that two separate pathways influence vector delivery in the nucleus. Using a siRNA approach, they found that the knockdown of nucleolar proteins had effects similar to those of Proteasome inhibition or HU, and increased transduction.

Through this work, researchers have refined the understanding of AAV2 trafficking dynamics and identified cellular parameters that mobilize virions in the nucleus and significantly influence AAV infection. This research will aid the development of successful vectors for therapeutic applications.

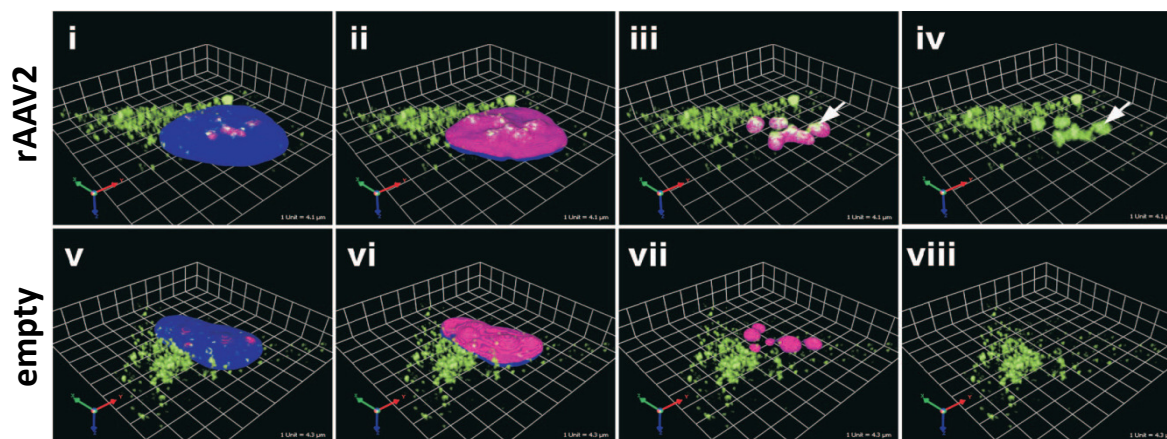


Figure: Accumulation of rAAV2 in the nucleolus
HeLa cells were infected for 16 h with either rAAV2 (i-iv) or empty capsids (v-viii) in the presence of a proteasome inhibitor, and immunofluorescence experiments were performed. 3D rendering of infection was performed using **Volocity**. The nucleus (blue channel) was gated to reveal nucleoplasmic staining of the nucleolar marker NCL (magenta) (ii and vi), focal nucleoli (iii and vii), and then the presence or absence of rAAV2 and empty capsids within nucleoli (yellow) (iv and viii). White arrows depict nucleolar accumulation.