Supporting Information

Electrophoresis-mediated Characterization of Full and Empty Adeno-Associated Virus Capsids

Adriana Coll De Peña[1], Lucy Masto[2], James Atwood[3], and Anubhav Tripathi[1,*]

[1]Center for Biomedical Engineering, School of Engineering, Brown University, Providence, RI, USA

[2]Division of Biology and Medicine, Brown University, Providence, RI, USA

[3]Applied Genomics, Perkin Elmer, Hopkinton, MA, USA

*Correspondence should be addressed to the following author:

Anubhav Tripathi (PhD)

Center for Biomedical Engineering, School of Engineering,

182 Hope Street, Providence, RI 02912, USA

Email: anubhav_tripathi@brown.edu

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Materials and Methods for Protein Charge Variant Experiments

For the AAV experiments below, the full AAV2 and AAV8 samples are Welgen (Welgen, Inc., Worcester, MA) null controls, at a stock concentration of 5 x 10^{12} VP/mL, while the empty AAV2 sample is the Vigene Biosciences (Vigene Biosciences, Rockville, MD) empty reference standard, with a stock concentration of 1.27 x 10^{12} VP/mL but concentrated using the Amicon (MilliporeSigma, Burlington, MA) 50 kDa Filter. The Please note that the exact concentration was not assessed in this section, and that the concentration of sample volume requirements of these experiments are greater than those required by ProteinExpress and PicoRNA. The detection system used in these experiments is the GX Touch II LabChip system (Perkin Elmer, Waltham, MA), and the assay followed was their protein charge variant assay with modifications to the sample labeling protocol (Perkin Elmer). The different pH mixtures were made following the protein charge variant user guide and using the provided pH 5.6 and pH 7.2 buffers.

Table S1. Effect of Different pH Conditions on AAV2 Differentiation

Table S1: Effect of different pH conditions on full and empty AAV2 differentiation. As seen in the table below, despite the changes in pH, there is no statistically significant difference between the migration time of the full and the empty AAV particles at a pH of 5.6 or 6.5 within our system.

pH	Sample	Migration time (s)
5.6	Full	38.46 ± 0.33
	Empty	38.61 ± 0.33
6.5	Full	57.38 ± 0.59
	Empty	57.36 ± 0.66

Figure S1. Electropherograms of AAV2 Full, Empty, and Combined Samples

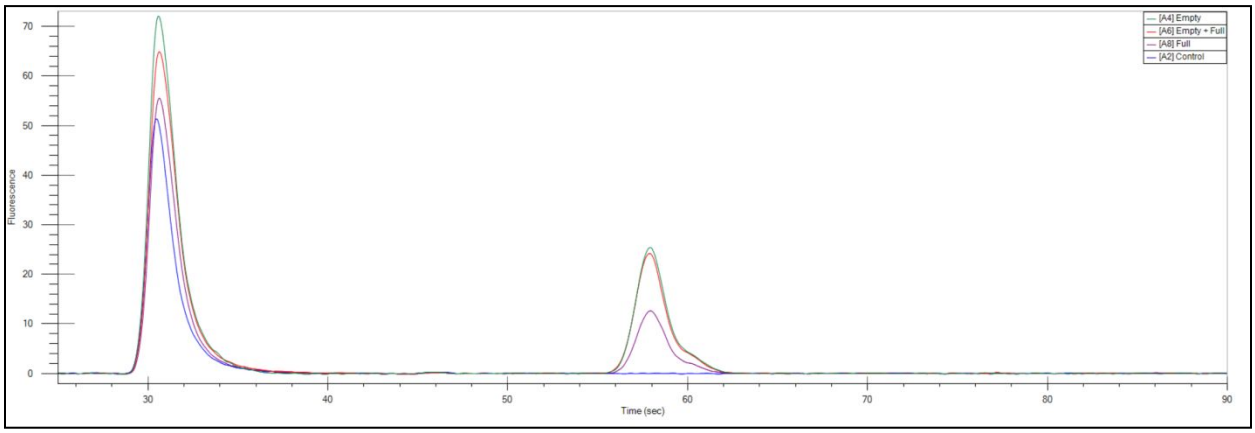

Figure S1: Electropherogram of control (blue), full (purple), empty (green), and empty + full (red) AAV2 samples analyzed at a pH of 6.5. The first peak (left to right) is the dye peak, present in the control which lacks any AAV particles, while the second peak is where AAV has been observed to migrate within our system. It must be noted that changes in dye peak magnitude have been observed across the experiments but are not believed to affect the results, and the changes in AAV peak magnitude are a result of differences in concentration between the samples. Moreover, this electropherogram is also a representation of the lack of differentiation or resolution between the empty and full sample peaks, as highlighted by the empty + full sample (red). A similar lack of differentiation was observed across all charge variant experiments regardless of the conditions or compounds used.

Table S2. Effect of Different Compounds on AAV2 Differentiation

Table S2: Effect of different compounds on AAV2 differentiation. Since changes in pH (Table S1 and Fig. S1) and in gel-matrix (experiments not included) did not differentiate full from empty AAV particles within our system, the next approach was to induce biochemical changes. Based on literature, antibodies (A1 and A69), digestion compounds (α-chymotrypsin, thermolysin, trypsin), as well as other compounds (PhosBind + streptavidin, urea, SDS) were used to see if they would induce a differential change or bind differentially to AAV in a manner that would result in a difference in mobility, represented by migration time in the table. However, despite the compounds and heat treatments used, we were unable to separate the full and empty peaks from each other. Interestingly, the only compound that appeared to significantly affect the mobility of AAV was α-chymotrypsin, and trypsin appeared to completely digest AAV at the concentration used in these experiments (0.02%) regardless of the incubation time (10-60 min). Please note that different temperatures and incubation times were followed for most compounds, but these were found to be the most representative for each compound.

Compound	Sample	pH	Heat treatment	Migration time (s)
Antibody A1	Full	5.6	65°C x 30 min	39.16 ± 0.02
	Empty			39.12 ± 0.19
Antibody A69	Full	5.6	65°C x 30 min	39.04 ± 0.11
	Empty			39.12 ± 0.19
α-chymotrypsin	Full	5.6	50°C x 30 min	32.34 ± 0.17
	Empty			32.33 ± 0.31
Thermolysin	Full	5.6	50°C x 30 min	38.51 ± 0.34
	Empty			38.58 ± 0.30
Trypsin	Empty	5.6	37°C x 10 min	No peak
	Empty			No peak
PhosBind + Streptavidin	Full	5.6	(RT) 25°C x 20 min	39.00 ± 0.03
	Empty			39.00 ± 0.03
Urea	Full	5.6	(RT) 25°C x 30 min	38.75 ± 0.08
	Empty			38.78 ± 0.07
SDS (under CMC*)	Full	5.6	50°C x 30 min	39.68 ± 0.45
	Empty			39.45 ± 0.36

* CMC = critical micelle concentration

Figure S2. Electropherograms of AAV2 and AAV8 Samples

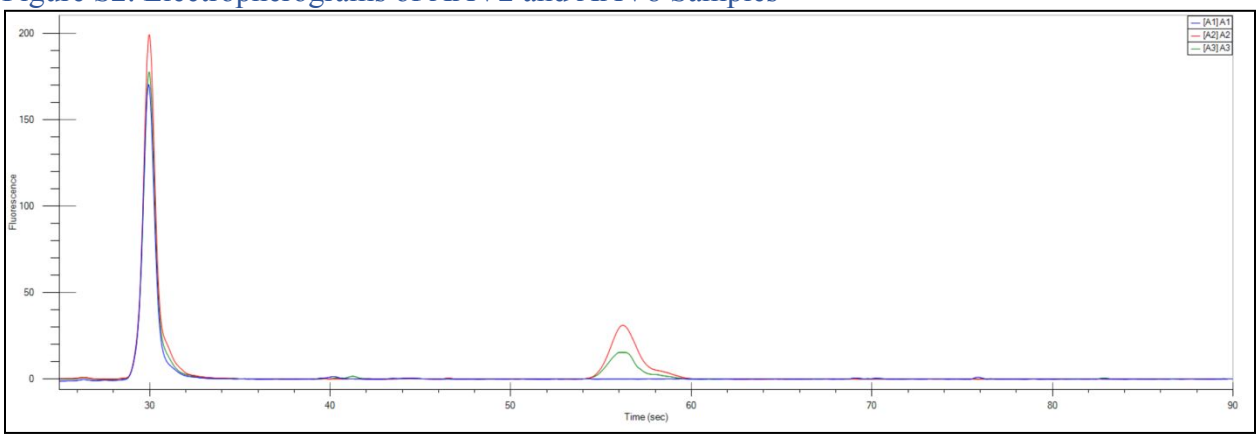

Figure S2: Electropherogram of control (blue), AAV2 (red), and AAV8 (green) samples analyzed at a pH of 6.5. The first peak (left to right) is the dye peak, present in all samples including the control which lacks any AAV particles, while the second peak is where AAV has been observed to migrate within our system. It must be noted that changes in dye peak magnitude have been observed across the experiments but are not believed to affect the results, and the changes in AAV peak magnitude are a result of differences in concentration between the samples or to differences in serotype interaction with the dye molecules. Moreover, this electropherogram is also a representation of the similarity of electrophoretic mobility profiles between AAV2 (peak center at 56.20 s) and AAV8 (peak center at 56.07 s). This similarity, which was also observed at different pH values, suggests the AAV2 results above may also be representative of AAV8 behavior. This is further supported by the fact that both samples are believed to contain a mixture of full and empty particles, which do not appear to be differentiated in this electropherogram. However, further studies need to be conducted prior to reaching additional conclusions.

Table S3. AAV9 compiled protein and ssDNA data collected and analyzed using mathematical prediction method

Table S3: The robustness of the proposed methodology was assessed by using it to predict the percentage of full capsids of a series of AAV9 samples made by combining an AAV9 full and empty reference standard at different ratios (Vigene Biosciences, Rockville, MD). Representative electropherograms for each condition are shown in Figure S3. The average prediction deviation for AAV9 was 3%.

Sample #	Percentage Full	Protein Area	DNA Area	Predicted Percentage Full	Prediction Deviation
1-0	82%	11.24 ± 0.23	18.78 ± 1.62	Standard	Standard
1-1	50%	7.41 ± 0.49	7.39 ± 0.77	49%	1%
1-2	25%	6.41 ± 0.32	2.56 ± 0.67*	20%	5%

*This value was estimated from duplicates whereas all others were estimated from triplicates due to the presence of the bubble in the detection channel at the time of analysis.

Figure S3. Electropherograms of AAV9 protein and ssDNA assays

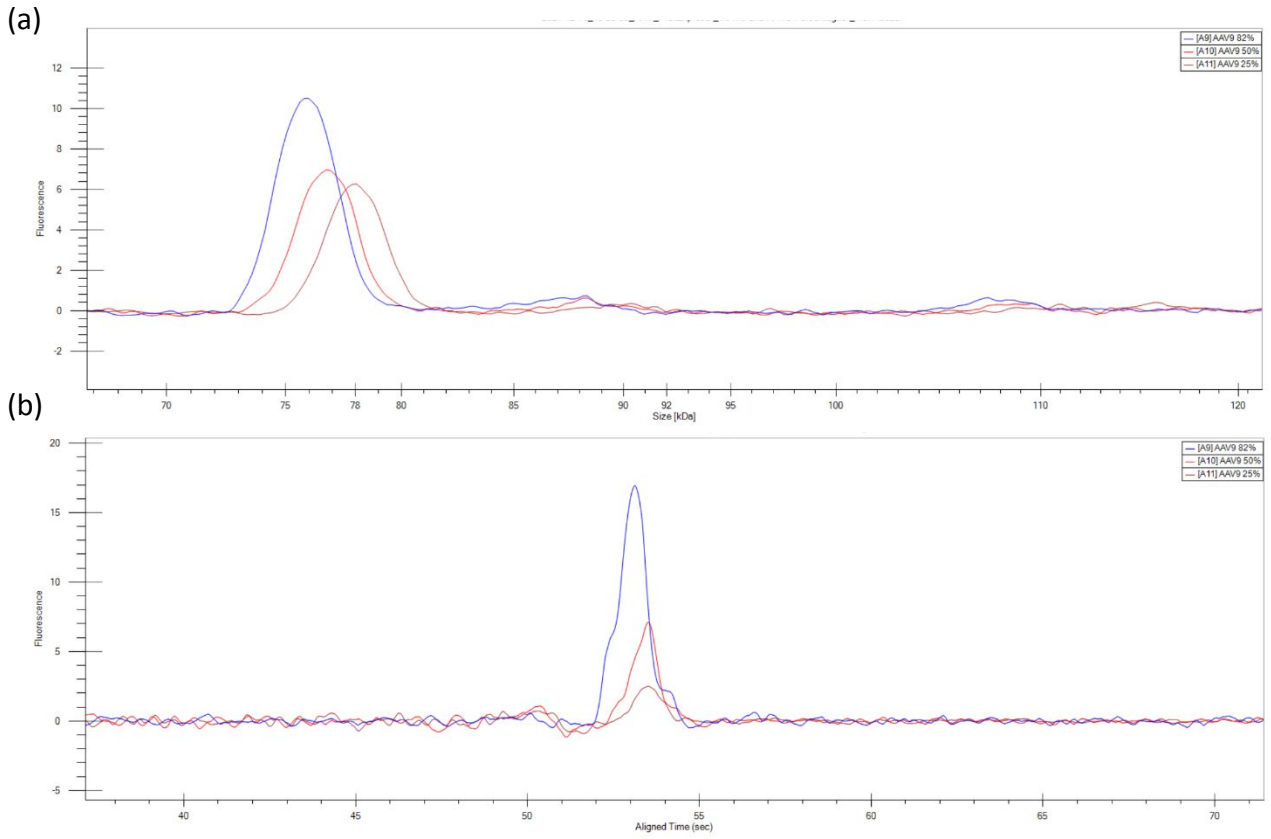

Figure S3: Representative AAV9 electropherograms of the (a) protein assay with the 82% full reference standard (blue), a 50% full sample (red) and 25% full sample (burgundy), and of the (b) ssDNA assay with the 82% full reference standard (blue), a 50% full sample (red) and 25% full sample (burgundy).