

Supplemental information:

1: Charge vs peak voltage

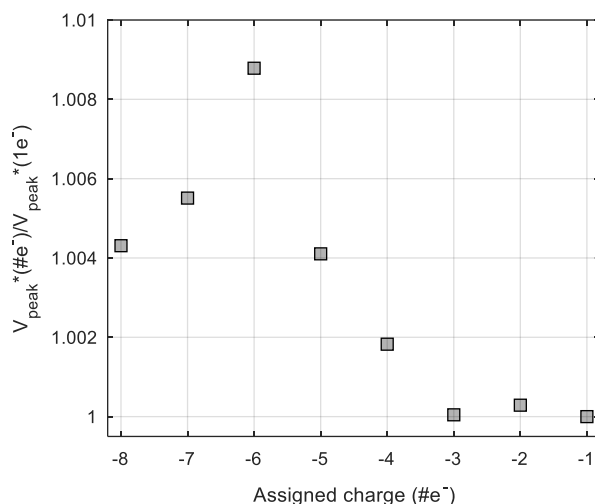


Figure SI1: Assigned charge vs peak DMA voltage comparison normalized by the voltage of the first peak and the number of charges for the various peaks of Figure 1B.

2) Relationship between DMA voltage and electrophoretic mobility diameter.

Corrections for humid air.

Humid air flow. Flowrate is measured with a SFM3013 (Sensirion AG). It is a hot wall thermal mass flow meter calibrated for dry air, flowrate is reported in units of SLPM for standard conditions of 20C and 1013mBar. For the case of humid air, the manufacturer provides a correction table as a function of temperature, pressure and humidity but it does not include the heat capacity ratio correction. For the conditions of 27C and 40% R.H, the partial pressure of water is 1.42kPa (1.4% gas fraction @1013mBar). Heat capacity of the mixture is 29.17 J/molK compared to 29.07 for dry air. Therefore, the flowmeter gas correction factor is: $G_{CF} = c_{p,w} / c_{p,d} = 1.0036$ where d and w reflect wet and dry conditions. The volumetric flow Q at the DMA is then corrected with the density ratio of dry to wet at standard conditions and at the experimental condition (wet@27C and dry@20C gases are 1.1695 and 1.2038 kg/m³ respectively.).

$$Q_{w,27C} = G_{CF} Q_{d,std} \rho_{d,std} / \rho_{d,27C}$$

Electrical mobility:

Electrical mobility is calculated as:

$$Z = \frac{Q_{sh}}{2\pi VL^*} \ln \frac{r_2}{r_1}$$

The Perez-DMA used in this study is Perez-MT “thin” (also named Perez-F) which shares almost the same channel geometry with the Perez-LT “thin”. These family of devices use a conical inner electrode for better aerodynamic performance, therefore the inlet to outlet distance L is corrected by the factor K into $L^*=LK$, details of the computation of K have been reported earlier (Perez-Lorenzo 2020). Q_{sh} is the sheath flowrate, L^* is the effective DMA length, r_1 is the diameter of the inner electrode at the outlet position L , r_2 is the diameter of the outer electrode. For the Perez-F DMA used the characteristics are as follows: $\alpha = 3$ degrees; $L = 11.48517$ (cm); $r_{1@L} = 1.00895$ (cm); $r_2 = 2$ (cm); $K_{theory} = 0.677162$;

Due to the lack of accepted mobility calibrants in the size range of interest, measurements of gas phase electrical mobility and therefore their corresponding mobility diameters may incur systematic error between instruments.

Electrical mobility to diameter conversion:

For the electrical mobility to diameter conversion since $m_p \gg m_g$, we use the modified Stokes-Milikan equation using $d_g = 0.3$ (Larriba¹):

$$Z_p = \frac{neC_c}{3\pi\mu(d_p + d_g)}$$

Where n is number of charges, e is the elementary charge, C_c is Cunningham slip correction factor², μ is the viscosity of the humid gas and d_p and d_g are particle and gas diameters respectively.

$$C_c = 1 + \frac{2\lambda}{(d_p + d_g)} (1.257 + 0.4e^{-0.55(d_p + d_g)/\lambda})$$

Using the collisional mean free path:

$$\lambda = \frac{\mu}{\rho} \sqrt{\frac{\pi m_g}{2k_B T}}$$

Where ρ is density, μ is the viscosity, k_B is Boltzmann's constant, T is temperature, and m_g is the molecular weight of the gas. For our conditions, adapted for the corresponding vapor loading of the humid air, $\lambda \sim 66.9\text{nm}$.

For each experiment the corresponding variables are updated for the actual experimental temperature. Then d_p is solved numerically for each experimental mobility reading.

Ultimately each datum can be converted to a mobility diameter.

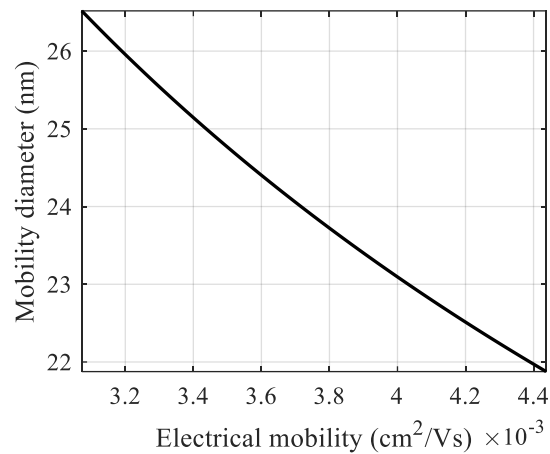


Figure SI2: Electrical mobility to diameter correlation for a characteristic experiment at the conditions of 40%R.H. and T = 27C.

3. Cargo content, concentration and length of the 12 AAV samples used in the study:

Table SI1: Cargo content, initial concentration and length of the 12 AAV samples used in the study. “Observed main peak” in mass and diameter results from identifying the peak in the spectra that most closely matches the nominal length.

Genomic cargo	Concentration (GC/mL)	Nominal length (kb)	Main peak observed CD-MS Molecular weight (MDa)	Main peak observed ES-DMA Mobility diameter (nm)
AAV9-Empty	$\sim 1.2 \cdot 10^{13}$	0.00	3.77	23.32
AAV9-CMV-Null	$\sim 5 \cdot 10^{13}$	1.80	4.36	23.87
AAV9-hSyn-eGFP.HA	$\sim 4 \cdot 10^{13}$	1.90	4.50	23.98
AAV9-CMV-mCherry	$\sim 3 \cdot 10^{13}$	2.20	4.57	24.04
AAV9-eSYN-RFP	$\sim 5 \cdot 10^{13}$	2.60	4.79	24.43
AAV9-CamKII(0.4)-TdTomato	$\sim 2 \cdot 10^{13}$	2.80	4.80	24.49
AAV9-EF1a-eGFP	$\sim 3 \cdot 10^{13}$	2.90	4.97	24.78
AAV9-CAG-eGFP	$\sim 3 \cdot 10^{13}$	3.30	4.98	24.80
AAV9-GFAP(0.7)-EGFP-T2AiCre	$\sim 1 \cdot 10^{13}$	3.60	5.06	24.99
AAV9-EF1a-EGFP-T2A-iCr	$\sim 3 \cdot 10^{13}$	4.10	5.20	25.22
AAV9-CAG-iCre/eGFP	$\sim 1 \cdot 10^{13}$	4.60	5.35	25.47
AAV9-CMV7-spCas9	$\sim 1 \cdot 10^{13}$	5.00	5.42	25.57

3: Effect of paraformaldehyde on mass and mobility

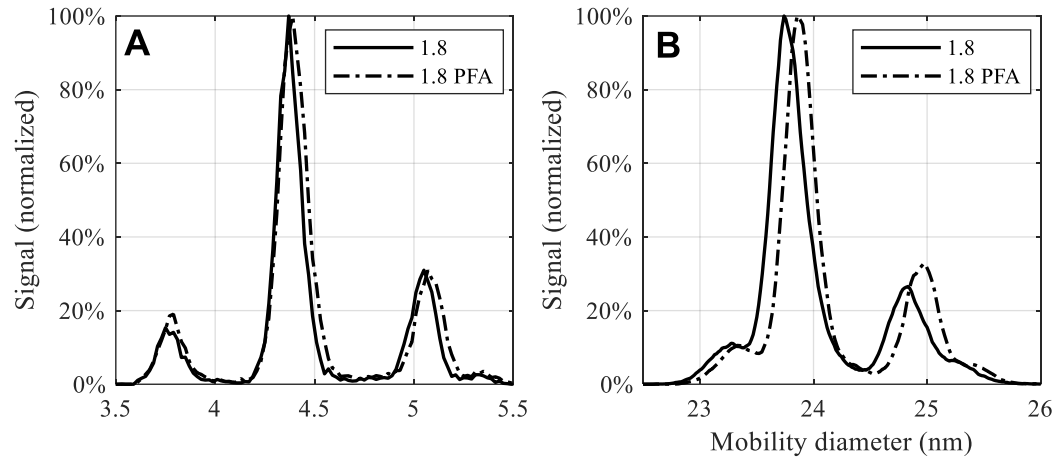


Figure S13. Mass (left) and diameter (right) comparison of AAV9 (1.8kbp) sample prepared with or without the PFA treatment. In both mass (left) and mobility (right) the PFA treated samples are shifted to heavier/larger (red mass, dot-dash diameter) with respect to the untreated cases (solid black)

4) Side by side ES-DMA comparison with CD-MS for the remaining subset of the 12-sample panel.

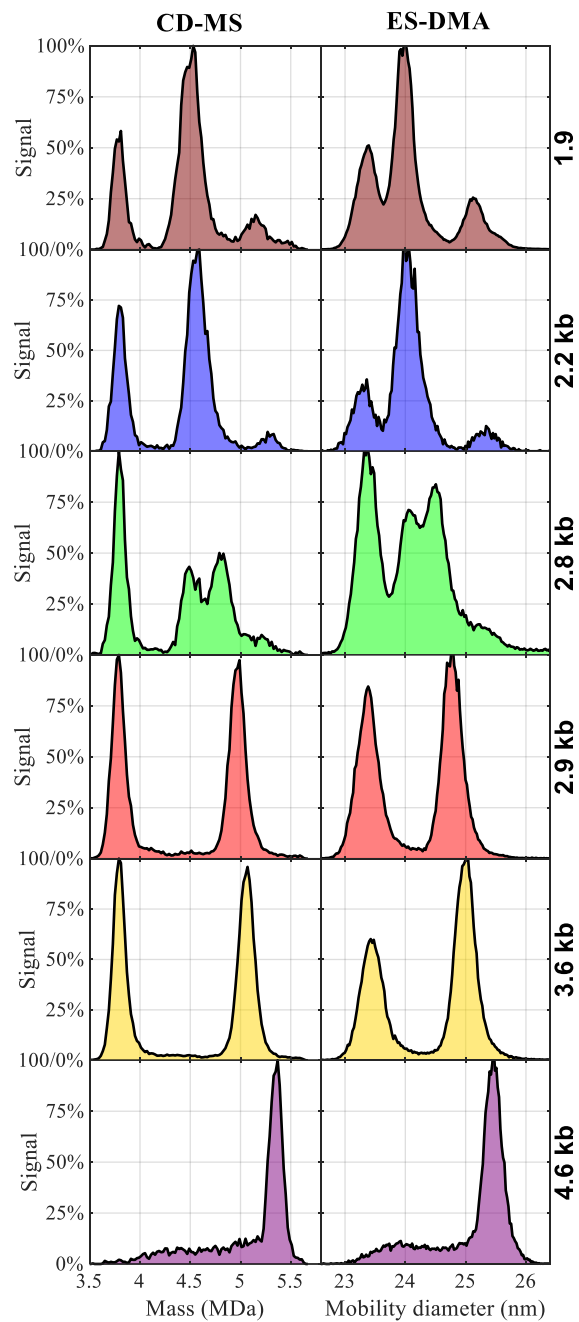


Figure SI4: – ES-DMA comparison with CD-MS reveals size / mass correlation for AAV9 capsids containing various length DNA cargo. For each row, left and right correspond with CD-MS and DMA respectively.

5) Gaussian fittings for the 12 AAV9 sample panel

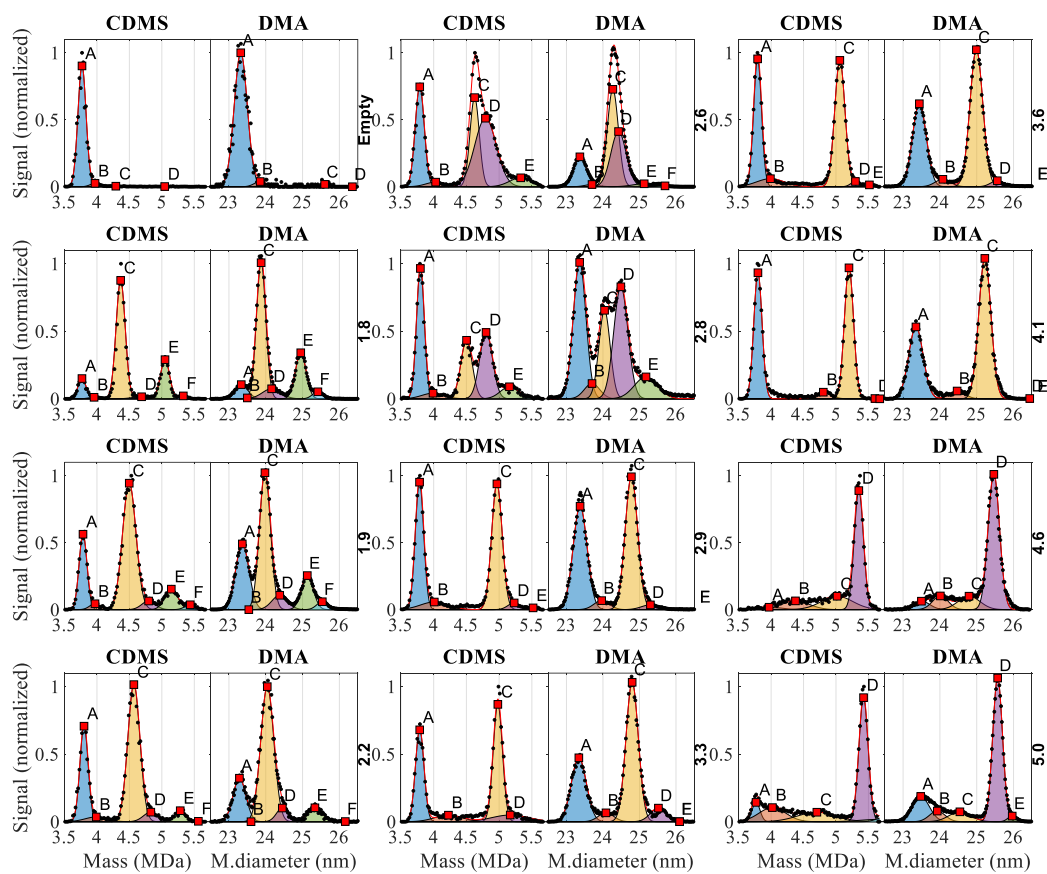


Figure S15: Analysis of the various samples with each peak assignment A through E ordinally ranked by ascending weight or diameter. The nominal cargo length of each case is indicated.

5) Relationship between the manufacturer stated *nominal cargo* and the observed nominal peak cargo and diameter.

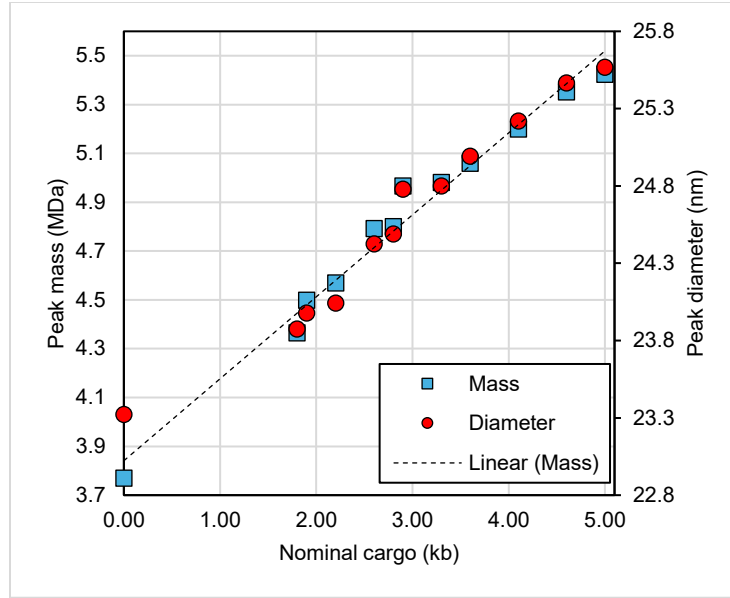


Figure S16: Relationship between the stated nominal cargo and nominal peak mass and diameter. The linear fit for mass-cargo results in the expression: $y = 0.3357x + 3.8411$, where x is cargo in (kb) and y is mass in MDa.

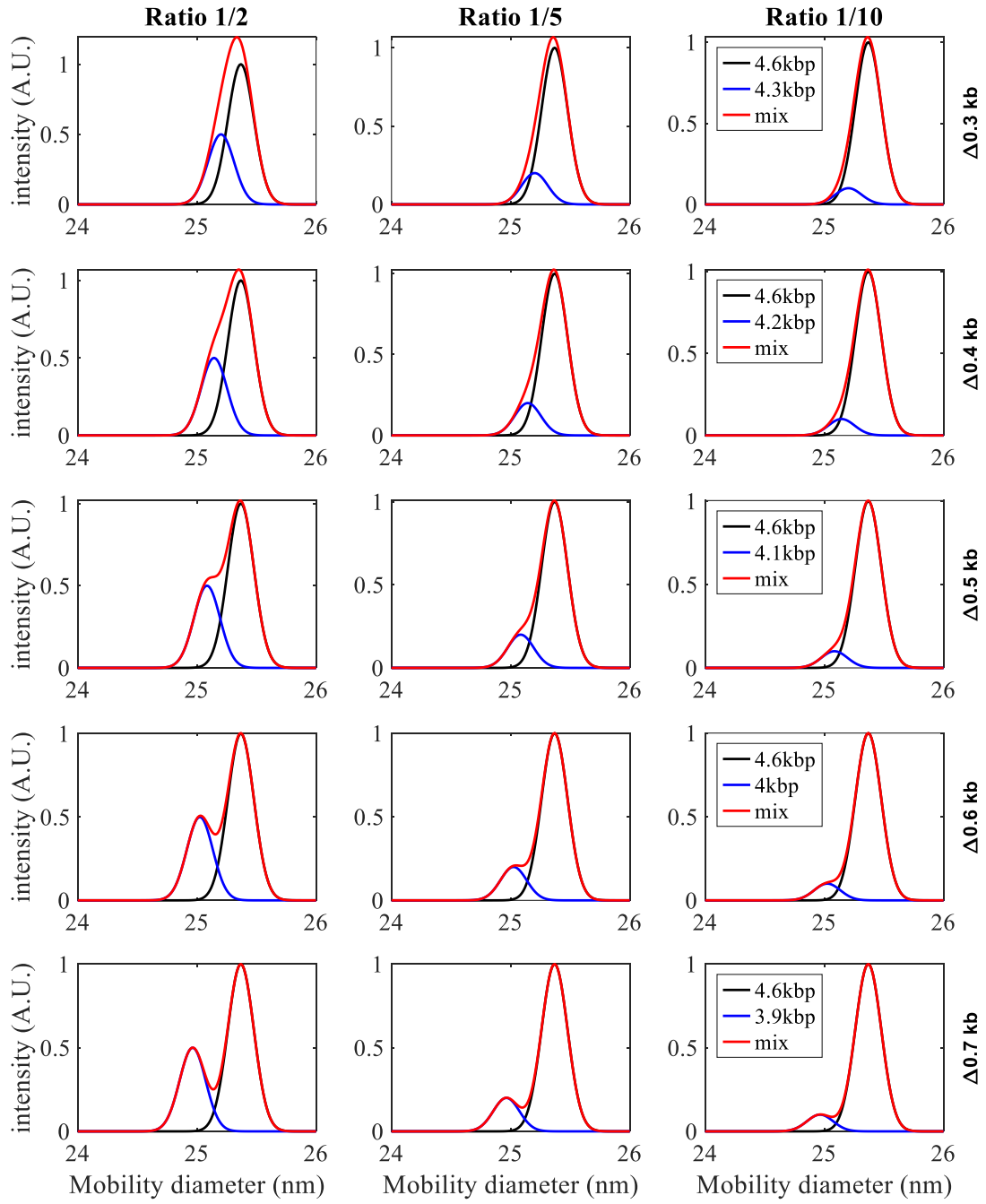


Figure SI7: Simulation of synthetic peaks

References:

- 1) Larriba, C., Hogan, C. J., Attoui, M., Borrajo, R., Garcia, J. F., & de la Mora, J. F. (2011). The Mobility–Volume Relationship below 3.0 nm Examined by Tandem Mobility–Mass Measurement. *Aerosol Science and Technology*, 45(4), 453–467.
<https://doi.org/10.1080/02786826.2010.546820>
- 2) Davies, C. N. Definitive equations for the fluid resistance of spheres. *Proc. Phys. Soc.* 1945, 57, 259-270.