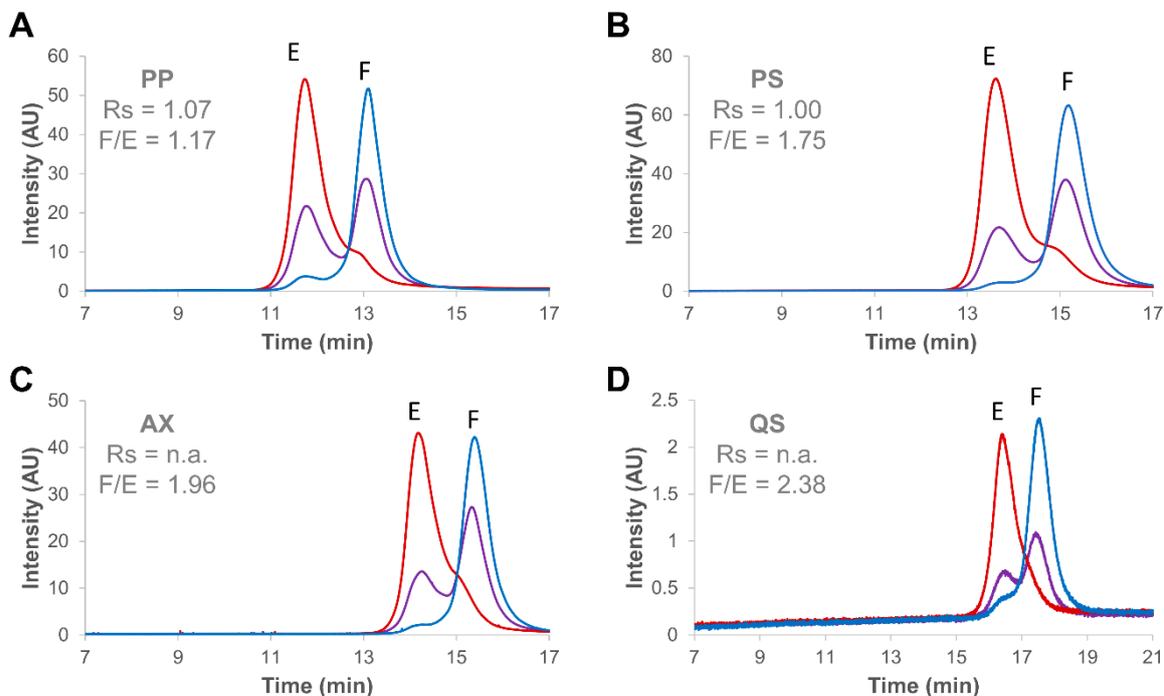


# Supporting Information

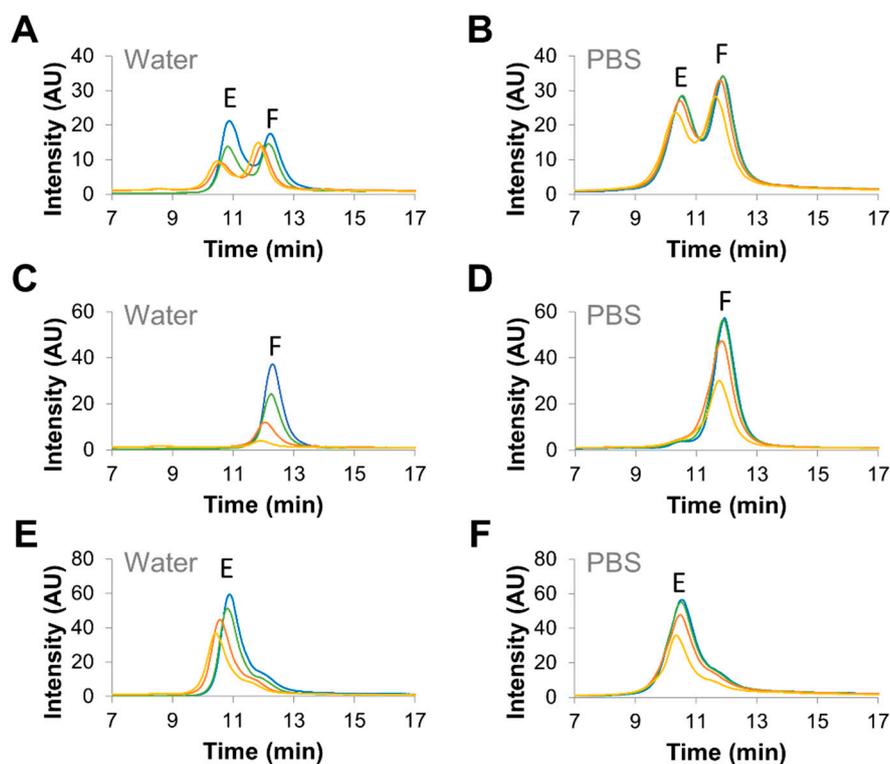
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**Figure S1.** Comparison of different column hardware. Empty (red line), full (blue line) and mixture of full and empty (violet line) rAAV8 capsids analysed in AEX mode by using 4 different column hardware, namely PP (A), PS (B), AX (C), and QS (D). Gradient conditions: reference linear gradient consisting of BTP buffer pH 9.0 in combination with NaCl as eluent salt. Acronyms legend: E = empty rAAV8 capsid, F = full rAAV8 capsid, Rs = peak resolution, F/E = full/empty ratio.



**Figure S2.** Sample stability study over 7 days. Samples consisting of full/empty mixture (panels A and B), full (panels C and D), and empty (panels E and F) rAAV8 capsids were diluted in water (panels A, C, E) and PBS buffer enriched with 0.001% poloxamer 188 (panels B, D, F) and analyzed at 0 (blue), 1 (green), 3 (orange), and 7 (yellow) days after dilution. Acronyms legend: E = empty rAAV8 capsid, F = full rAAV8 capsid.



Summary of the chromatographic parameters related to the rAAV8 stability study:

	$t_R$ empty (min)	$t_R$ full (min)	F/E ratio	Area empty (AU)	Area full (AU)	Resolution
<b>Dilution in water</b>						
Day 0	10.88	12.23	0.89	21.21	17.55	1.03
Day 1	10.83	12.17	1.06	13.85	14.62	1.09
Day 3	10.60	11.95	1.37	9.11	14.04	1.08
Day 7	10.47	11.84	1.38	9.60	15.02	1.08
SD	0.19	0.18	0.24	5.60	1.55	0.03
Average	10.69	12.05	1.18	13.44	15.31	1.07
RSD (%)	1.8%	1.5%	20.5%	41.7%	10.1%	2.5%
<b>Dilution in PBS buffer</b>						
Day 0	10.51	11.87	1.09	28.40	34.24	n.a.
Day 1	10.55	11.87	1.09	28.56	34.18	n.a.
Day 3	10.47	11.78	1.12	27.01	33.03	n.a.
Day 7	10.32	11.64	1.17	23.75	28.38	n.a.
SD	0.10	0.11	0.04	2.23	2.78	/
Average	10.46	11.80	1.12	26.93	32.46	/
RSD (%)	0.9%	1.0%	3.4%	8.3%	8.6%	/

**Figure S3.** Screening of mobile phase flow rate. Mixture of full and empty rAAV8 capsids analysed in AEX mode by using three different flow rates, namely 0.7 mL/min (grey line), 0.3 mL/min (green line), and 0.1 mL/min (orange line). Gradient conditions: optimized linear gradient consisting of AMPSO buffer pH 9.4 in combination with TMAC as eluent salt.

