Supporting Information

Surface Charge Dictates the Mechanism of Cellular Uptake of Fluorescent Amine Passivated Carbon Dots

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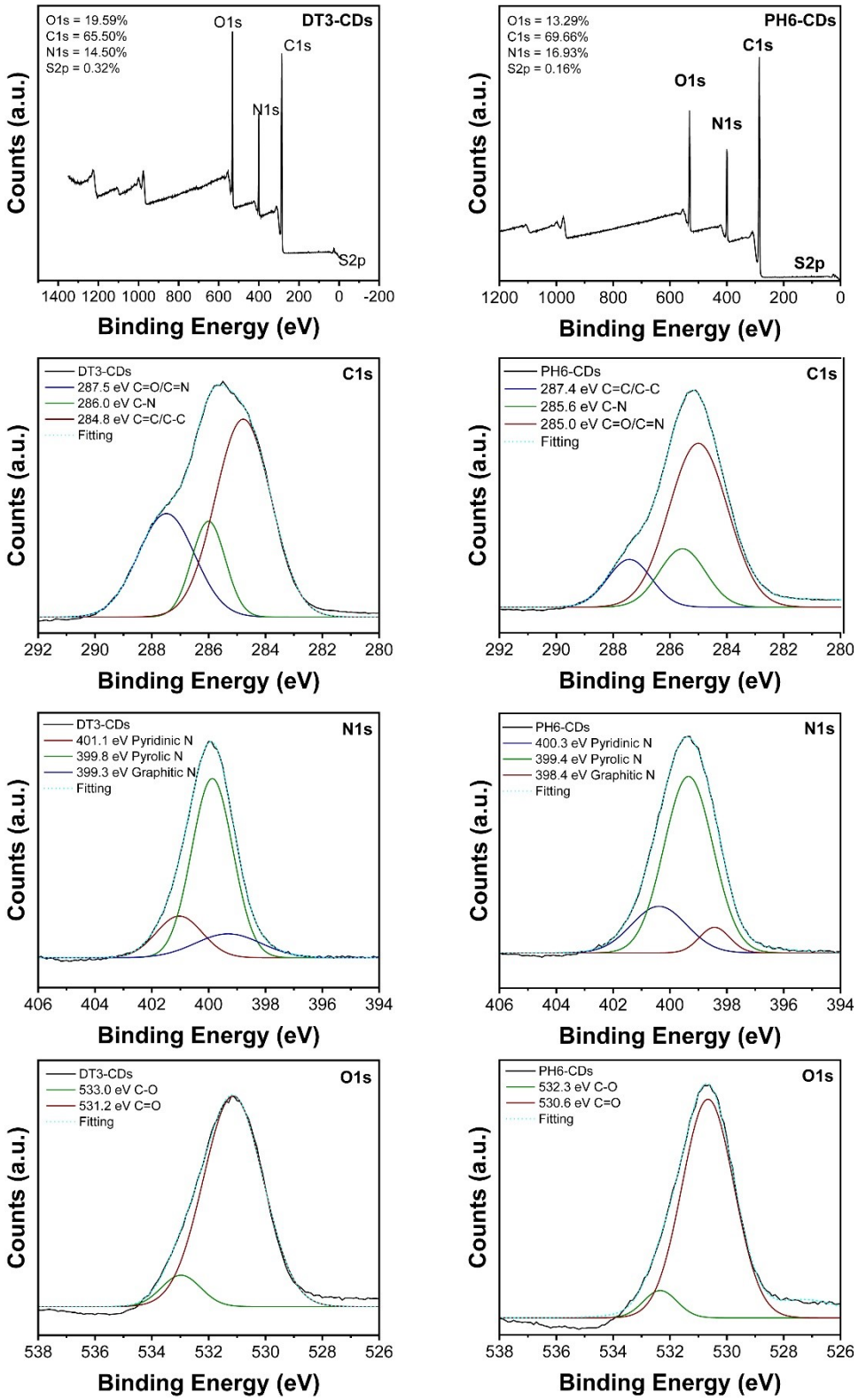

Figure S1. XPS survey and deconvoluted HR-spectra of all CDs showcasing 3 binding energies ascribed to C1s, N1s and O1s for DT3-CDs (left) and PH6-CDs (right)

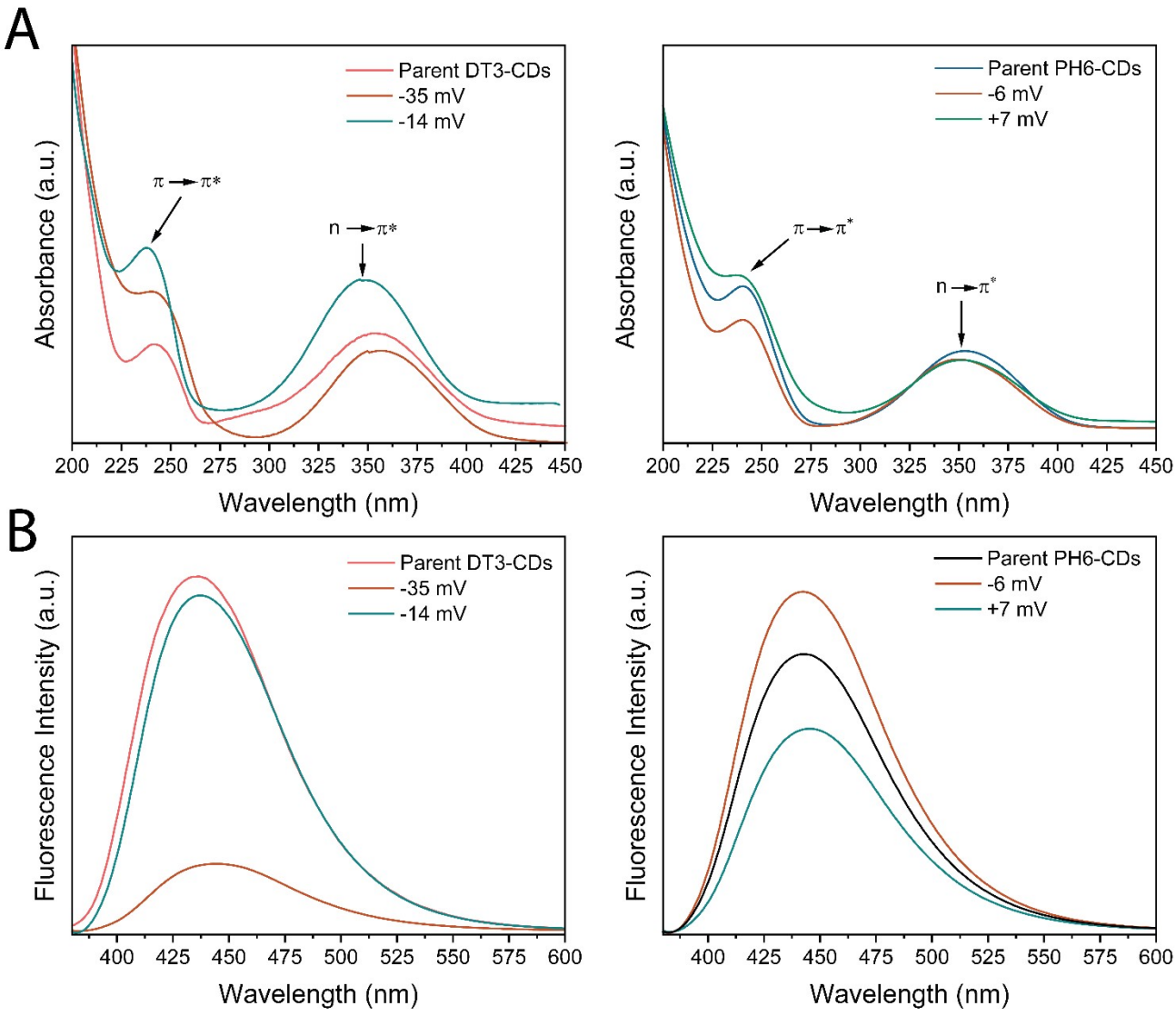

Figure S2. Optical properties of charge separated CDs. (A) Room temperature absorbance spectra of the charge separated CDs and the parent CDs in an aqueous solution. The left panel showcases DT3-CDs and the right panel showcases PH6-CDs. The UV-Vis absorption spectrum the CDs reveals two absorption bands centered at 240 nm and 335–355 nm, which reflect the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition, respectively. (B) Room temperature fluorescence spectra of the charge separated CDs in an aqueous solution. Following maximum excitation at 350 nm one emission maxima is measured at 440 nm for DT3-CDs and 445 nm for PH6-CDs.

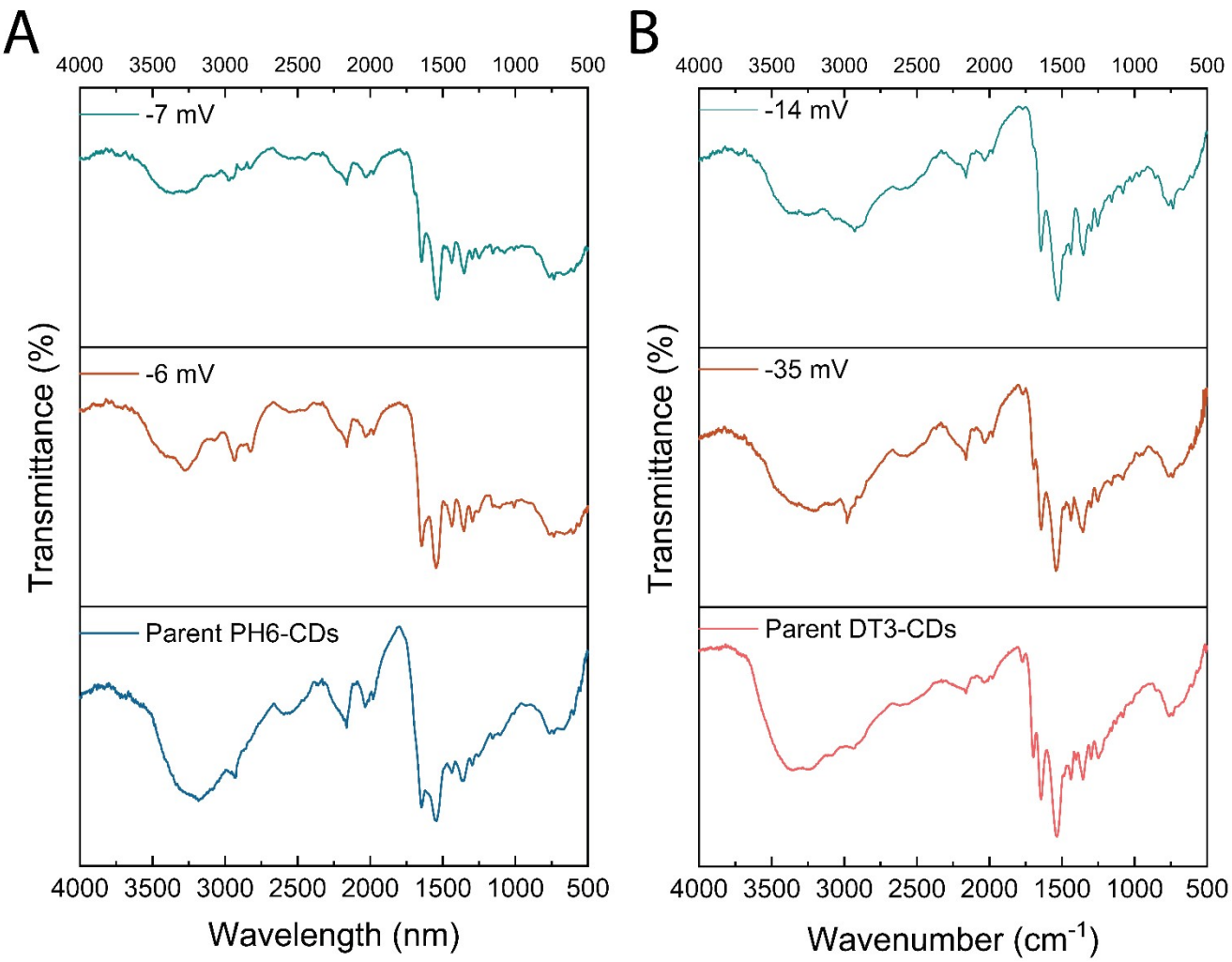

Figure S3. Surface functional groups of charge separated CDs. A graph shows the stacked FT-IR spectra of PH6-CDs, PH6-CD (+) and PH6-CDs (-). The broad O-H stretch at 3250 cm^{-1} indicates a carboxylic acid functionality, and sp^3 and sp^2 C–H stretches can be seen at 2964 cm^{-1} and 2830 cm^{-1} respectively. Lastly, amide C=O, C=C/C=N, and N-H/C-N peaks appears at 1646 cm^{-1}, 1536 cm^{-1} and 1354 cm^{-1}. (B) Stacked FT-IR of the parent DT3-CDs, DT3-CDs (+) and DT3-CD (-).

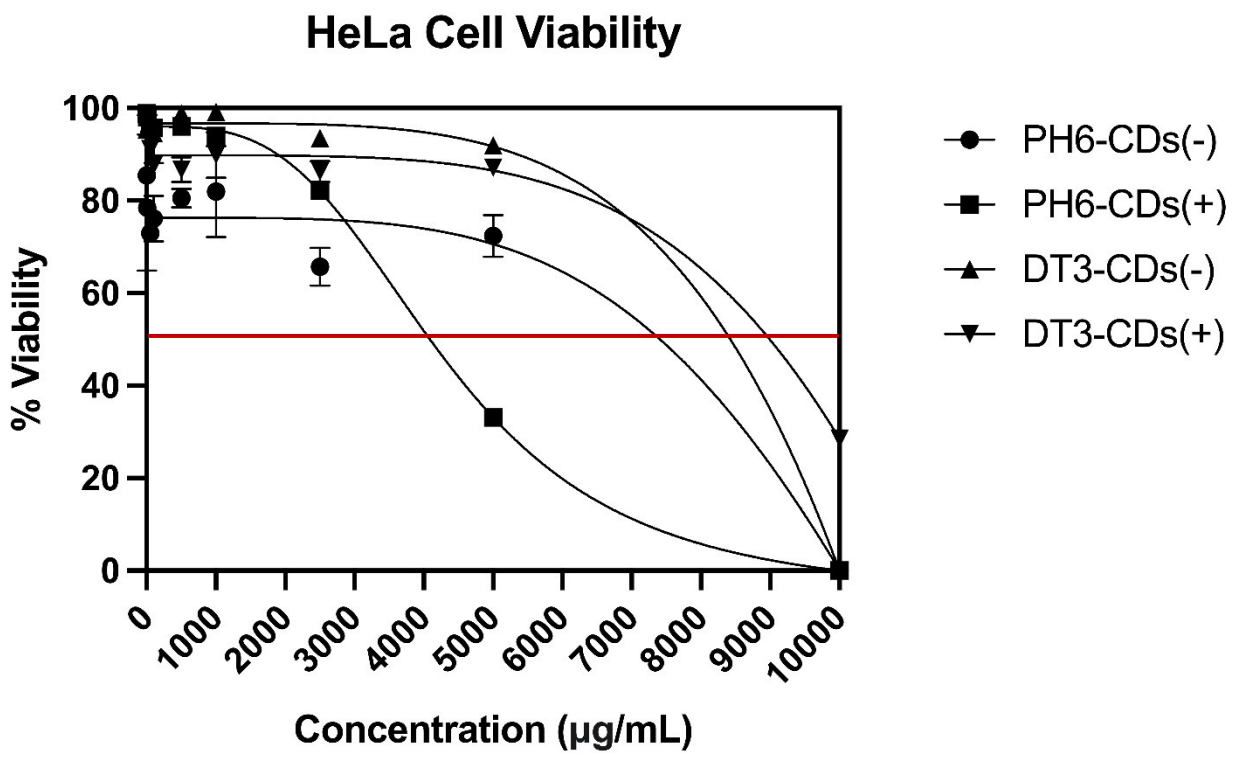

Figure S4. Cell viability of HeLa cells after treatment DT3-CDs and PH6-CDs. The graph shows the IC50 (dotted red line) for HeLa cell viability, after treatment with varying concentrations of the different CDs for three population doubling times (4200 µg/mL for PH6-CDs (+), 7500 µg/mL for PH6-CDs (-), 8500 µg/mL for DT3-CDs (+), and >10,000 µg/mL for DT3-CDs (-).

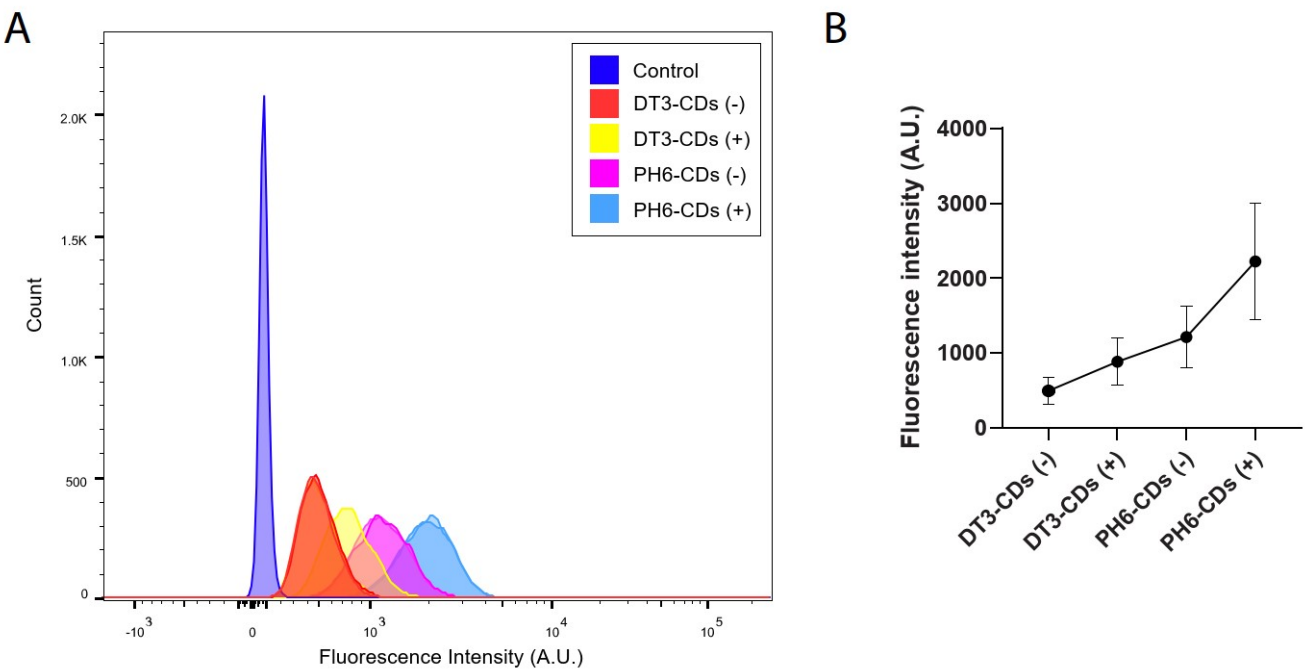

Figure S5. Flow Cytometry Analysis. (A) The histogram graph shows the fluorescence intensity distribution for HeLa cells after treatment with 500 µg/mL of DT3-CDs (+), DT3-CDs (-), PH6-CDs (-), and PH6-CDs (+) for 24 hours. 10,000 cells per sample (n=2). (B) The graph on the left shows the mean fluorescence intensity of CDs treated HeLa cells, whiskers represent the coefficient of variation. Both graphs highlight the positive correlation between surface charge and cellular uptake.

Table S1. One-Way ANOVA and Paired t-test statistical analysis for cellular uptake and cytosolic level of CDs in HeLa cells for Fig.3

Statistical Test		Groups	P-value	Data Interpretation
Mean Intensity	One-way ANOVA	DT3-CDs (-) DT3-CDs (+)	4.92E-20	Population means are significantly different
	Paired t-test	DT3-CDs (+): DT3-CDs (-)	4.00E-8	Population means are significantly different
Cytosolic Intensity	One-way ANOVA	DT3-CDs (-) DT3-CDs (+)	3.62E-37	Population means are significantly different
	Paired t-test	DT3-CDs (+): DT3-CDs (-)	1.24E-8	Population means are significantly different

Table S2. One-Way ANOVA and Paired t-test statistical analysis for cellular uptake and cytosolic level of CDs in HeLa cells for Fig.4

Statistical Test		Groups	P-value	Data Interpretation
Mean Intensity	One-way ANOVA	PH6-CDs (-) PH6-CDs (+)	0.01	Population means are significantly different
	Paired t-test	PH6-CDs (+): PH6-CDs (-)	0.00	Population means are significantly different
Cytosolic Intensity	One-way ANOVA	PH6-CDs (-) PH6-CDs (+)	0.38	Population means are NOT significantly different
	Paired t-test	PH6-CDs (+): PH6-CDs (-)	0.12	Population means are NOT significantly different

Table S3. One-Way ANOVA and Paired t-test statistical analysis for cellular uptake and cytosolic level of CDs in HeLa cells for Fig.5

Statistical Test		Groups	P-value	Data Interpretation
Mean Intensity	One-way ANOVA	DT3-CDs (-) DT3-CDs (+) PH6-CDs (-) PH6-CDs (+)	3.43-34	Population means are significantly different
	Paired t-test	DT3-CDs (+): DT3-CDs (-)	1.96E-8	Population means are significantly different
		PH6-CDs (+): DT3-CDs (-)	0.00	Population means are significantly different
		PH6-CDs (-): DT3-CDs (-)	0.00	Population means are significantly different
Cytosolic Intensity	One-way ANOVA	DT3-CDs (-) DT3-CDs (+) PH6-CDs (-) PH6-CDs (+)	3.43E-34	Population means are significantly different
	Paired t-test	DT3-CDs (+): DT3-CDs (-)	0.00	Population means are significantly different
		PH6-CDs (+): DT3-CDs (-)	0.97	Population means are NOT significantly different
		PH6-CDs (-): DT3-CDs (-)	0.99	Population means are NOT significantly different