Supplementary Information

Nitrogen doped carbon dots for in vitro intracellular redox modulation via optical stimulation

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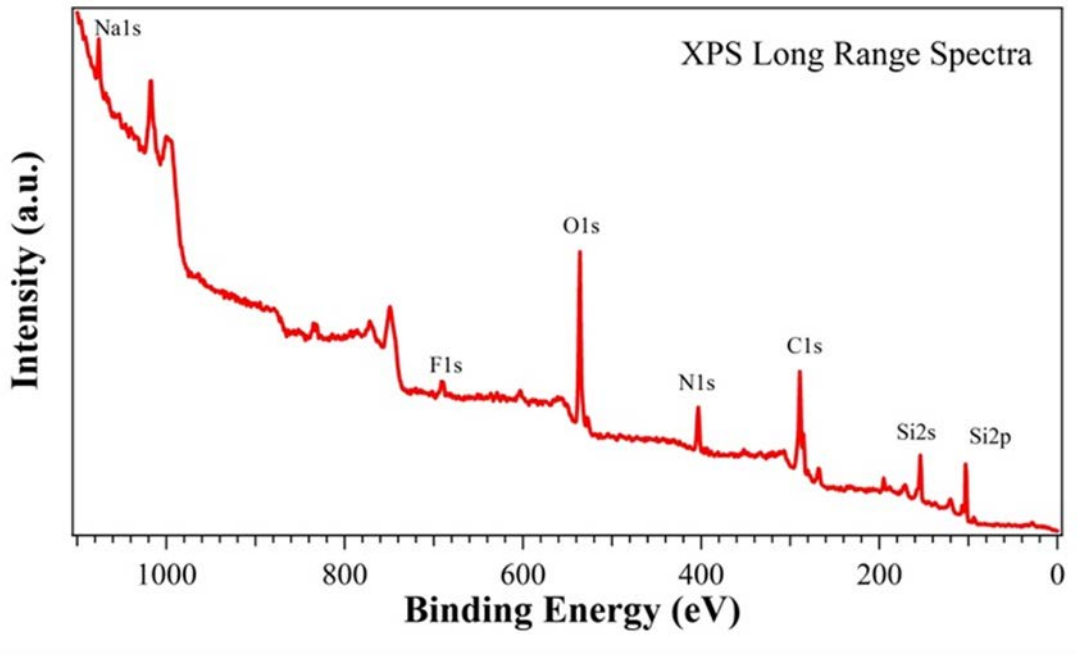


Figure S1. Full-scan XPS survey spectra of CDs deposited on silicon substrate

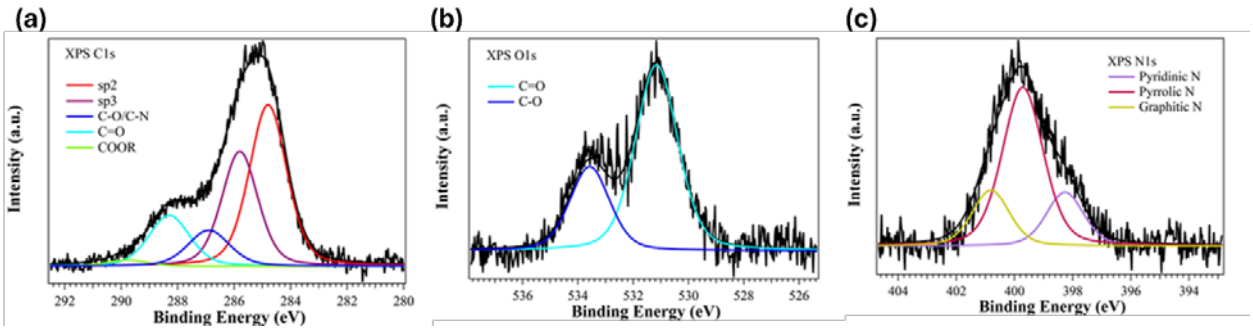


Figure S2. De-convoluted High Resolution XPS Spectra @ Pass Energy = 20 eV; (a) C 1s; (b) O 1s; (c) N 1s.

XPS spectra quantitative analysis

Long range spectrum shows the expected chemical species (carbon, oxygen and nitrogen, silicon) and some minor species (sodium and fluorine) in very low quantities. The atomic percentages are calculated considering the signal from the whole surface (CDs, contaminants and silicon substrate); the contribution from CDs only resulted 76.6% of C, 11.6% of N and 11.8% of O.

Core level analysis

Lineshape analysis has been carried out using Voigt lineshape after the subtraction of a Shirley background. The G-L ratio is 30%. C1s has been deconvoluted into five contributions taking into account the different species: sp^2 carbons (284.8 eV, main peak), sp^3 carbons (+1.0eV), C-N and or C-O bonds (286.9 eV), C=O bonds (288.3 eV), COOR bonds (289.8 eV). Accordingly, O1s shows two main contributions, that pairs with those found on C1s: O-C bonds at 533.6 eV and O=C bonds at 531.2 eV. N 1s lineshape has been represented using three main features: pyridinic N at 398.3 eV, pyrrolic N at 399.7 eV and graphitic N at 400.8 eV. The weight of each component over the relative core level signal is reported in the following, **Table S1.**

C1s	sp2	sp3	C-O/C-N	C=O	COOR
	43.3	29.5	10.6	14.8	1.8
O1s	O-C	O=C			
	29.9	70.1			
N1s	Pyridinic	Pyrrolic	Graphitic		
	18.9	61.5	19.6		

Table S1. Relative weight of each component for C 1s, O 1s, N 1s, according to deconvoluted High-Resolution XPS Spectra.

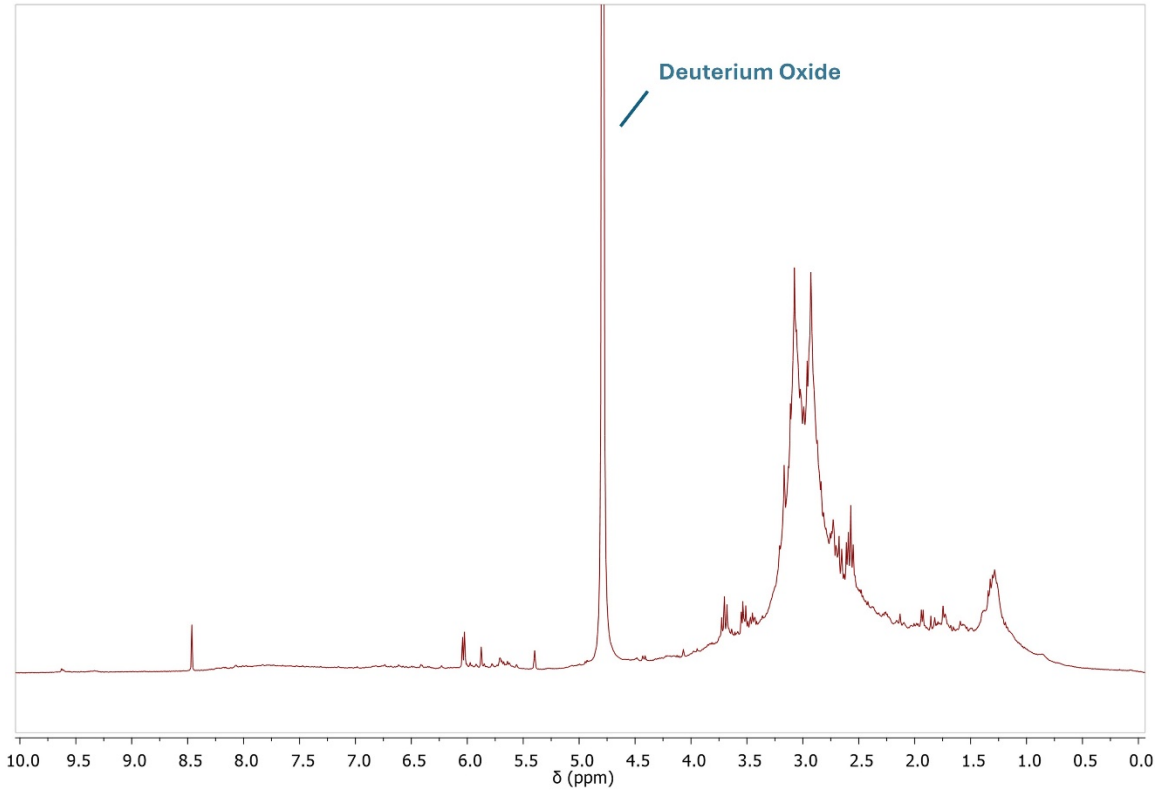


Figure S3. ^{1}H NMR spectrum of CDs recorded in D_2O.

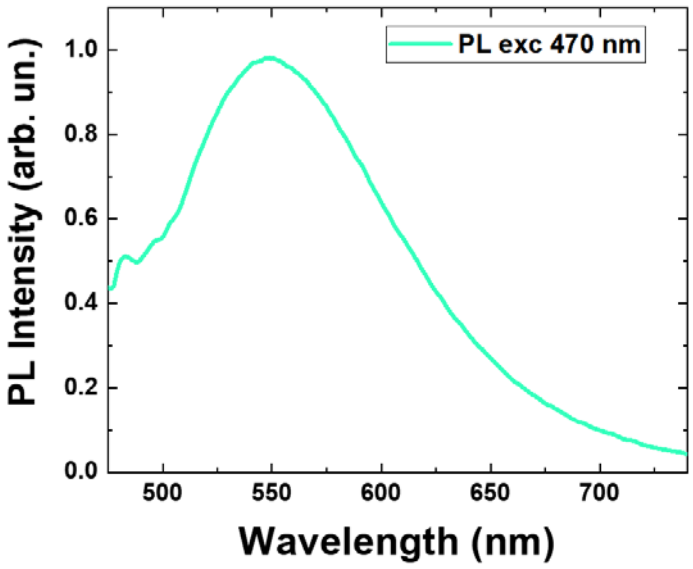


Figure S4. N-CDs emission spectrum upon 470 nm excitation.

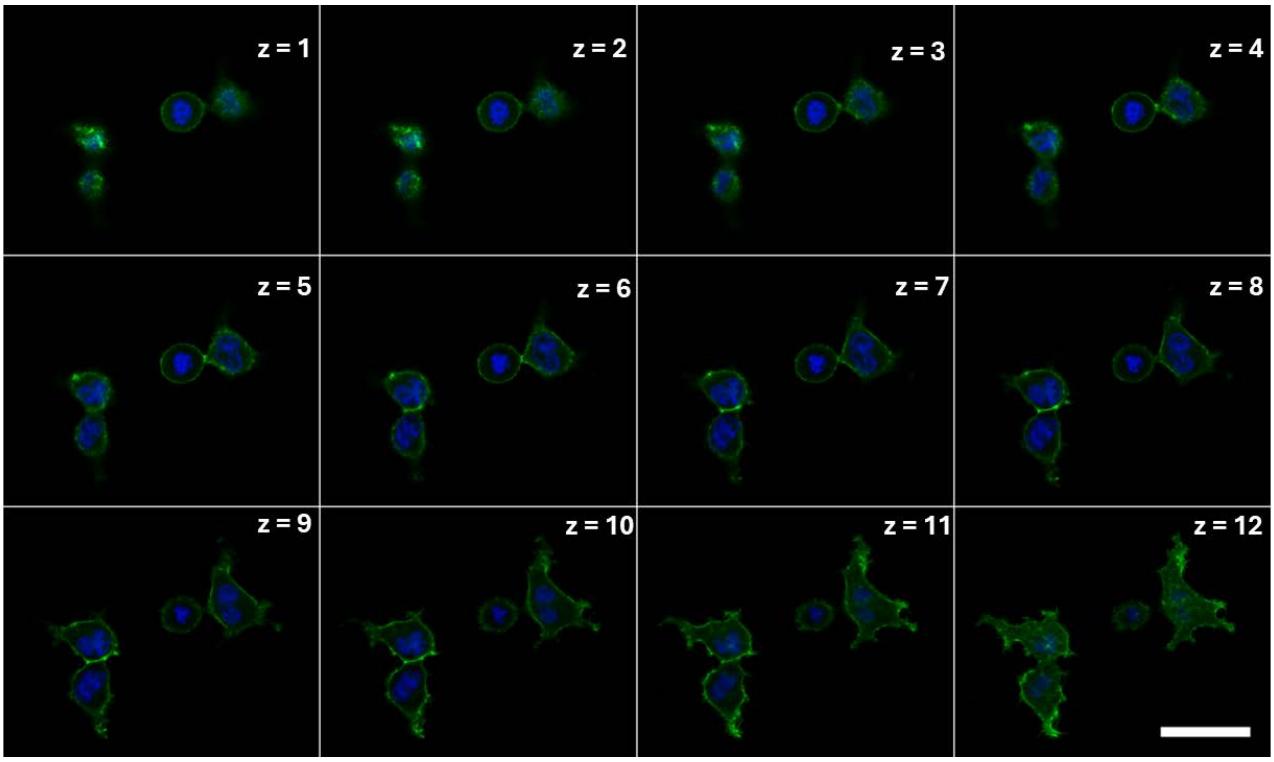


Figure S5. Representative confocal images of untreated HEK cells acquired at different z planes from cell top surface (upper left) to cell bottom (lower right). Cells are stained with Cell mask (cell membrane, green) and Hoechst 33342 (cell nuclei, blue). Images were acquired from top (z=1) to bottom (z=12). Interplane distance = 0.15 µm. Scale bar, 20 μm.

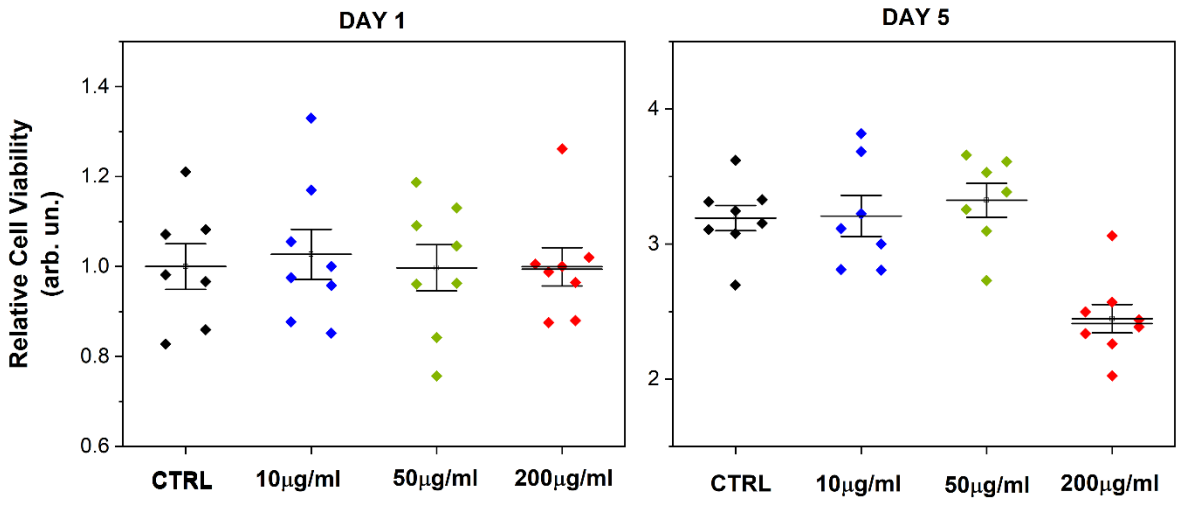


Figure S6. MTT cell viability assay on in vitro cell cultures treated with CDs concentration in the range 10 – 200 µg mL^{-1}, at 1 (left) and 5 (right) days in vitro (DIV) after plating. Data have been normalized to untreated control samples.

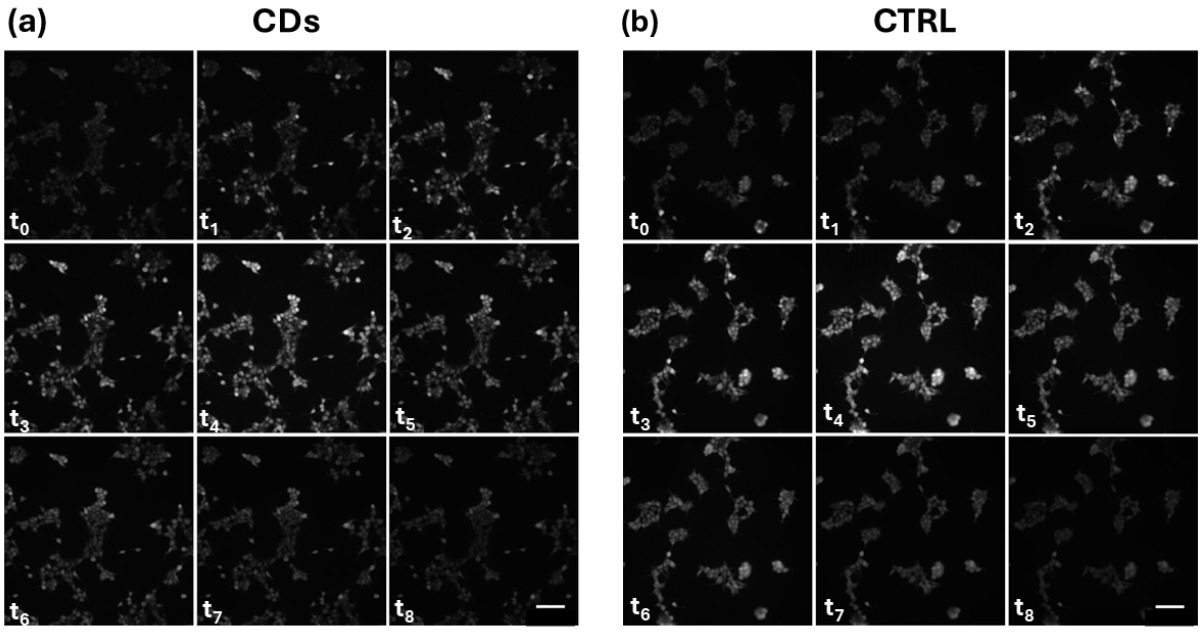
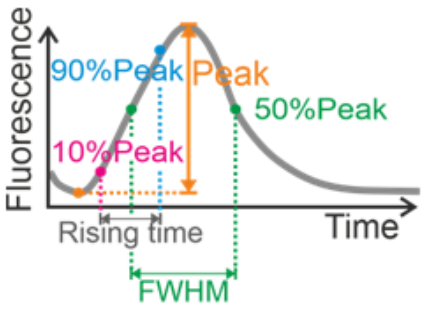
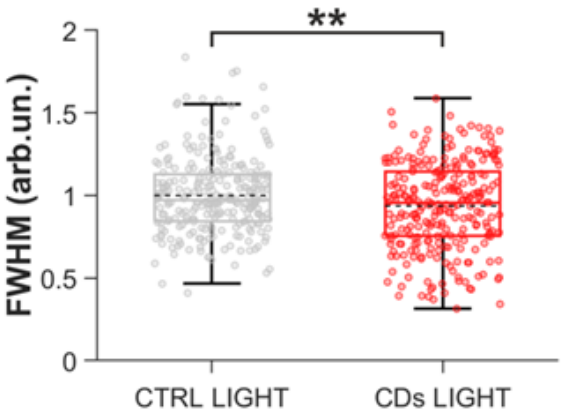


Figure S7. Representative fluorescence images of HEK cells treated with Ca^{2+} sensitive Fluo-4 fluorescent probe, in presence (a) or in absence (b) of CDs. The images were acquired at different time points, from t_0 (0 s) to t_8 (120 s). No detrimental optical overlap between CDs and Fluo-4 is observed, indicating that Fluo-4 excitation is not altered by the presence CDs. Scale bar, 10 μm.

(a)



(b)



(c)

Biological Replicate	Peak Amplitude (arb. un.)		Build-up Time (s)		Decay Time (s)		FWHM (s)	
	CTRL	CDs	CTRL	CDs	CTRL	CDs	CTRL	CDs
I	229	269	25	21	46	47	43	42
II	430	781	14	11	42	33	27	21
III	231	260	22	21	46	47	37	39

Figure S8. (a) Schematics of the main parameters extracted for Ca^{2+} transient curve analysis **(b)** FWHM of the Ca^{2+} transients recorded in untreated and CD-treated HEK cells (grey and red, respectively). Student's t-test, P-values: ** for $p < 0.01$; **(c)** Table showing mean values of Peak Amplitude, Build-up Time, Decay Time and FWHM across the three different biological replicates.