Carbon Nanodots: Opportunities and limitations to study their biodistribution at the human lung epithelial tissue barrier

Running title: The biodistribution of Carbon NanoDots in a lung model

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Table SI: Elemental analysis of CNDs

Samples	C%	H%	N%	S%	O% (calculated)
***N,S*-CNDs**	42.99	4.65	6.49	4.03	41.84
***N*-CNDs-1**	47.00	4.42	16.32		32.26
***N*-CNDs-2**	42.67	7.16	15.63		34.54

Elemental analysis show the successful doping of N,S-CNDs with nitrogen and and sulfur and the N-CNDs-1 and N-CNDs-2 with nitrogen.

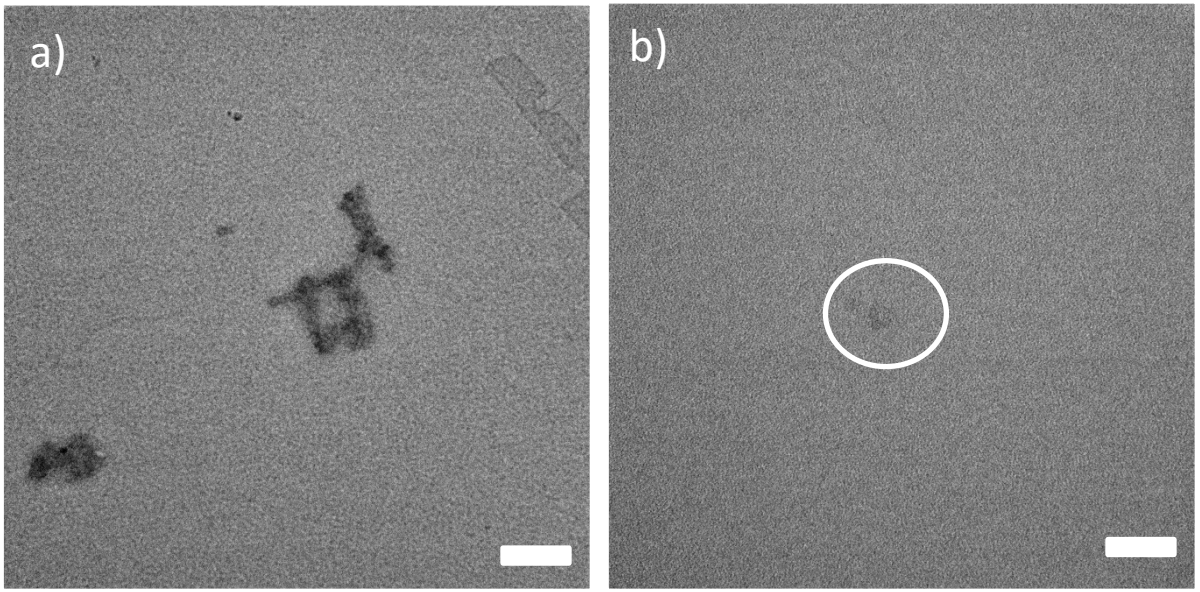

FIGURE S1: Representative TEM micrographs of agglomerated N-CNDs-1 proving the low contrast of the particles, when smaller agglomerates occur (b). a) scale bar: 200 nm, b) scale bar 50 nm.

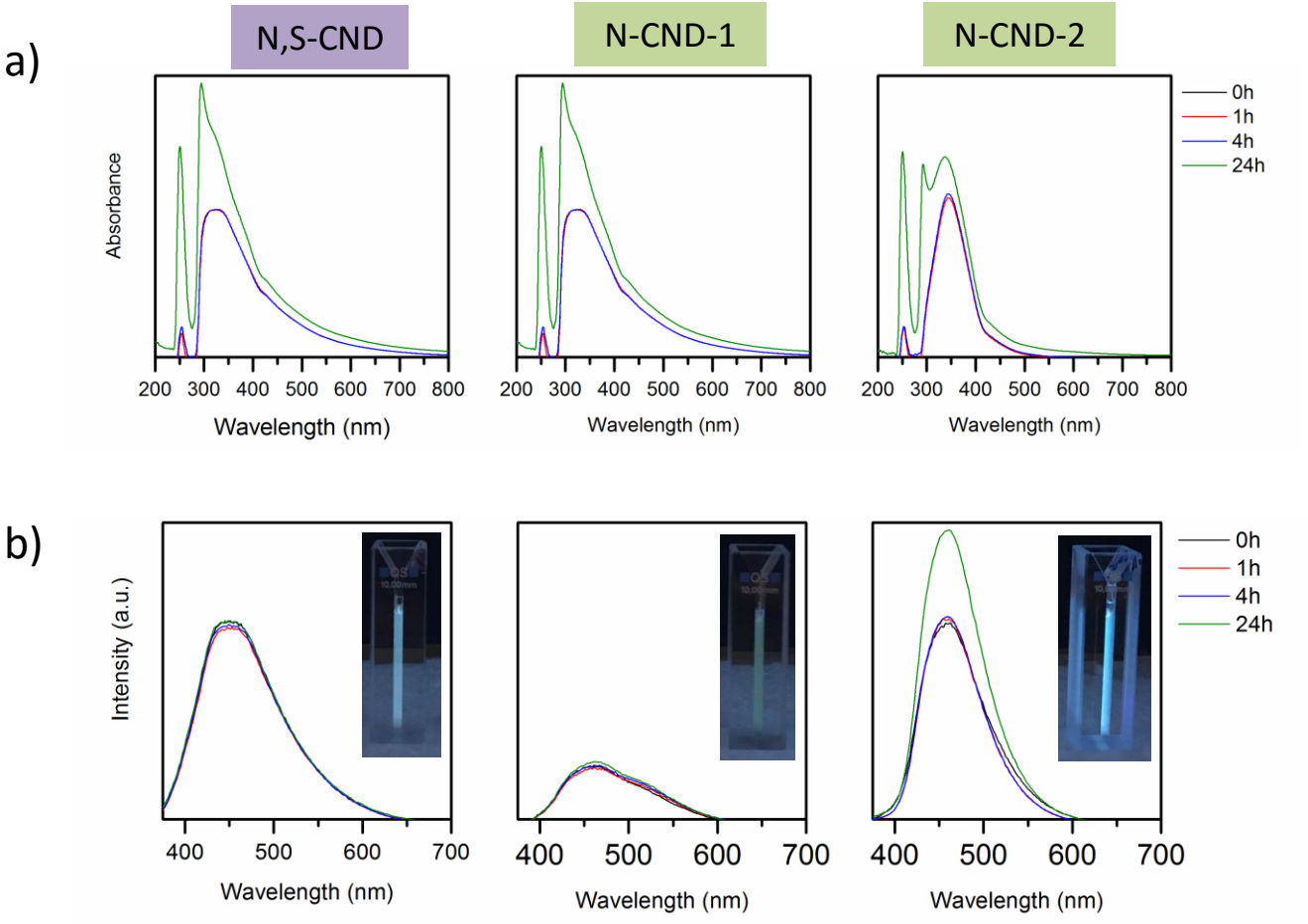

FIGURE S2: (a) UV-Vis and (b) fluorescent spectra of all three types of CNDs after 0, 1, 4 and 24 h at concentration 100 µg/mL in cDMEM. Images of CNDs in supplemented FluoroBrite DMEM under UV lamp (b) were taken after 24 h.

CNDs are stable in supplemented FluoroBrite DMEM until 4h and UV-Vis properties changed at 24h; however, the fluorescent signals remain equal. TDA results show stable size over 24 h.
N-CND-2 were diluted to 10 µg/mL to avoid saturation signal; signal at 24h is more intense due to evaporation.

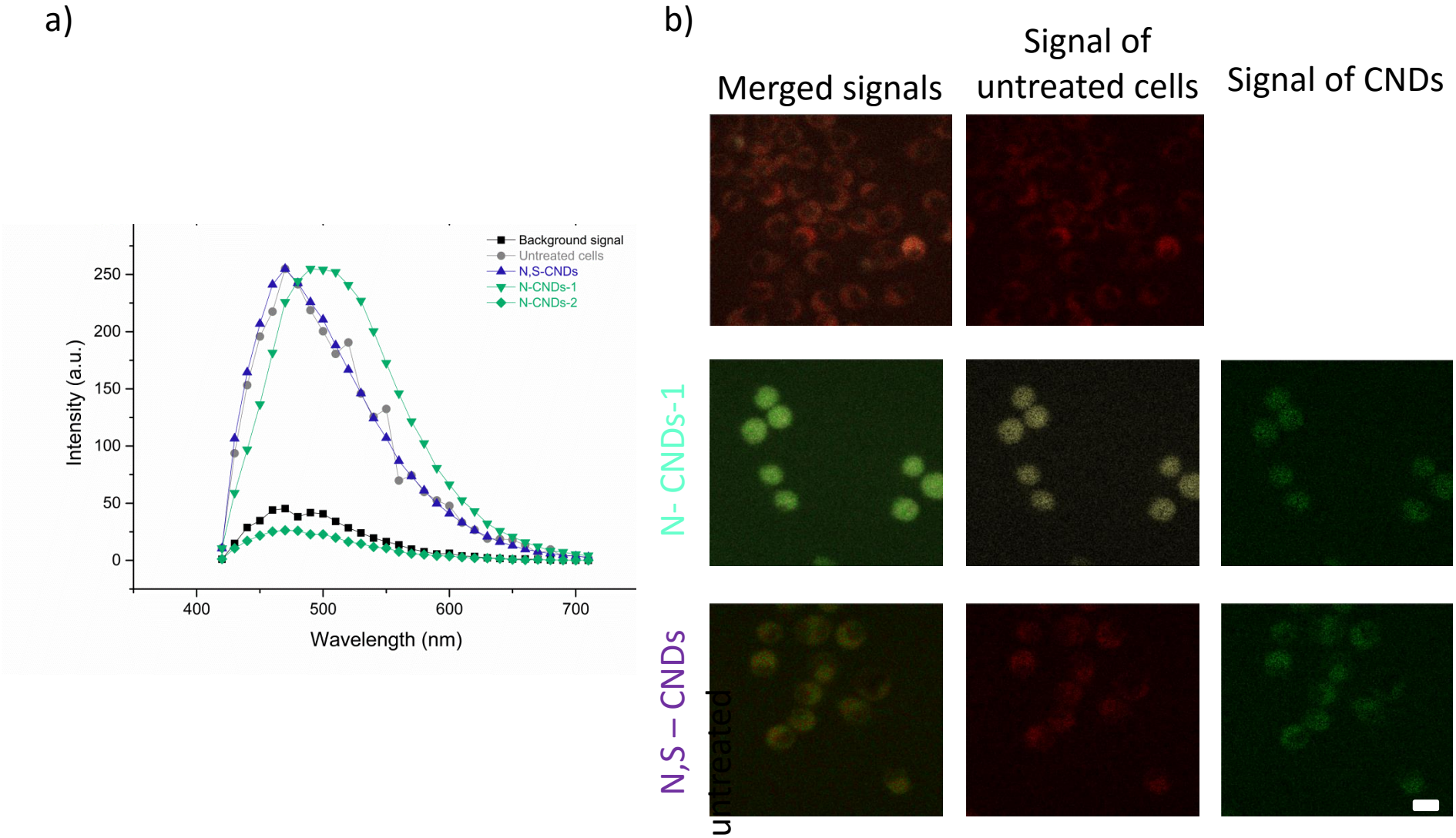

FIGURE S3: a) Spectra obtained using lambda mode in confocal microscope presenting overlapping of the untreated cells (J774A.1 mouse macrophage cell line used to improve the potential uptake) peak with CNDs peaks, (b) representative LSM images of unteated cells and cells treated with CNDs applying obtained spectra. Scale bar: 20 µm.