

Supporting Information

Engineered Anionic and Cationic Lignin-g-PLGA Nanoparticles for Controlled Delivery of Nano CuS in Lettuce (*Lactuca sativa*): Effects of Charge on Nutrient Uptake and Metabolic Activity

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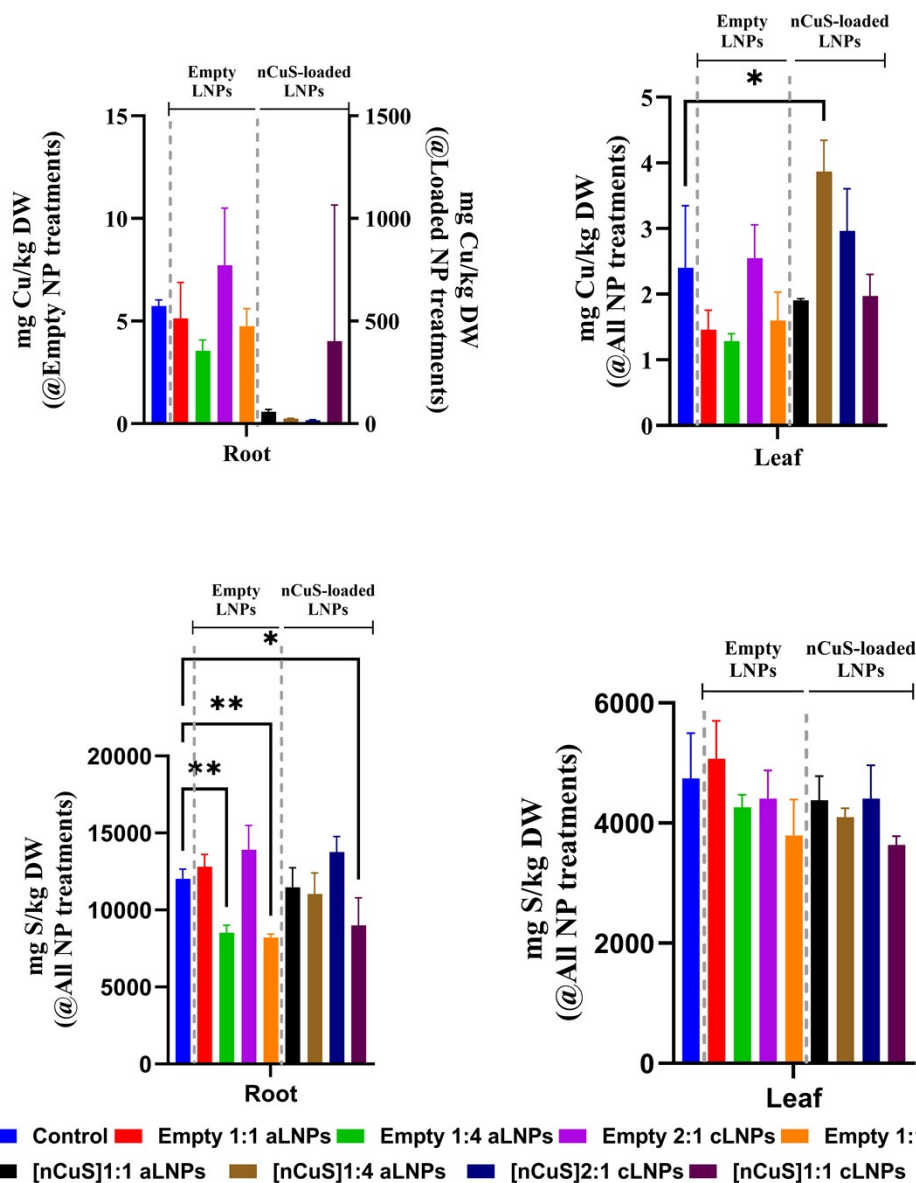


Fig. S 1 Cu and S concentrations of dry lettuce leaf and root tissue exposed to 50 mg/L of empty and nCuS-loaded anionic and cationic NPs under hydroponic cultivation. The data represent mean + SD (n = 3) with significant differences determined by one-way ANOVA followed by Dunnett's post-hoc test. (* = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001)

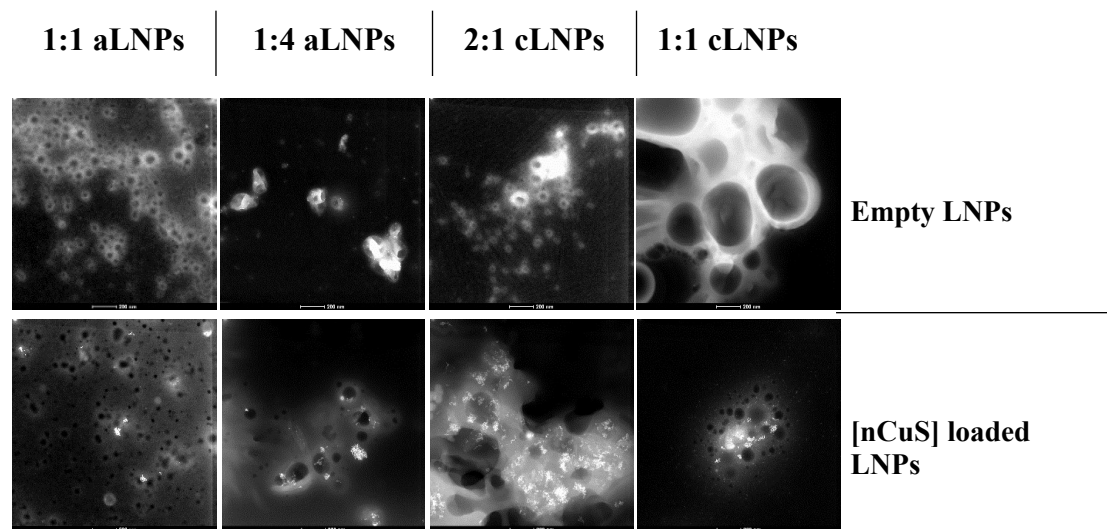


Fig. S 2 High-resolution S/TEM imaging showing spherical core-shell structures of empty and nCuS-loaded anionic and cationic nanoparticles at 56kx magnification. All scale bars represent 200 nm in the HAADF images.

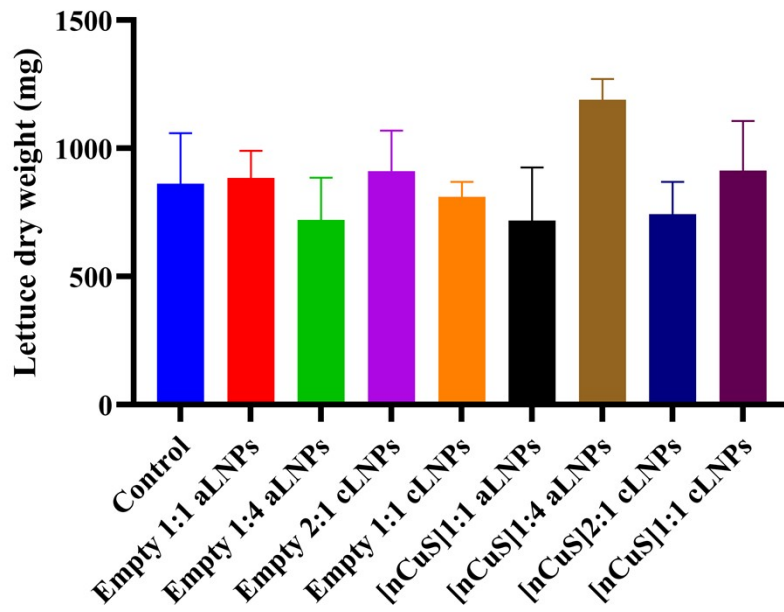


Fig. S 3 Lettuce biomass reported as dry weight showing no significant difference from the control (n=3)

Table S 1 Recipe of nutrient solution adapted for the hydroponic growth of lettuce plants

Chemical name	Molar mass M (g/mol)	Volume of solution (L)	Mass of chemical for a 1 L volume of nutrient solution
Potassium nitrate	101.1	1	202.206
Potassium silicate	154.28		128.616
Potassium dihydrogen phosphate	136.09		54.43
Calcium nitrate tetrahydrate	236.15		354.225
Magnesium sulfate heptahydrate	246.48		197.184
Nitric acid	63.01		63.013 (41.73 μL^{**})
Boric acid	61.83		2.473
Ethylenediaminetetraacetic acid, iron (III) monosodium salt (EDTA)	367.05		3.56
2-(N-morpholino) ethane sulfonic acid	213.2		976.2
Copper (II) sulfate			No Cu here
Prepare the following chemicals and add them all to 100 mL of distilled water. In order to make the complete Hoagland's solution, you must add 1 mL of this solution to the 1 L solution above			
Manganese (II) chloride tetrahydrate	197.9	0.1	59.37
Zinc (II) chloride	136.28		40.89
Nickel (II) chloride hexahydrate	237.71		1.296
Sodium molybdate nonahydrate	240.18		2.06
1/ Adjust pH to 5.8 – 6.0			
2/ Stir properly and check pH			

Table S 2 Leaf humidity and leaf temperature of leaves of hydroponically grown and nanoparticle-treated lettuce

Parameter	Plant leaf	Treatment								
		Control	Empty 1:1 aLNPs	[nCuS]1:1 aLNPs	Empty 1:4 aLNPs	[nCuS]1:4 aLNPs	Empty 2:1 cLNPs	[nCuS]2:1 cLNPs	Empty 1:1 cLNPs	[nCuS]1:1 cLNPs
Leaf humidity (RH%)	Outer leaves	45.3±3.4	43.3±4.6	42.6±3.4	42.4±4.4	45.8±4.3	43.4±3.3	46.4±4.8	43.9±43.9	48.2±5.4
	Middle leaves	53.8±4.3	53.1±5.9	50.8±4.2	51.3±6.3	52.8±3.2	47.6±4.1**	50.3±3.2	50.6±4.2	52.8±5.4
	Inner leaves	51.9±3.0	50.6±4.2	50.7±3.9	46.9±3.3**	52.0±3.3	47.3±2.7*	53.9±4.1	53.4±4.8	51.6±3.3
Leaf temperature (°C)	Outer leaves	21.7±0.8	23.3±0.1	23.3±0.3	25.0±0.1	25.3±	25.3±0.1	25.8±0.4	24.8±0.1	24.9±0.1
	Middle leaves	21.8±0.8	23.3±0.1	23.3±0.3	25.0±0.1	25.3±0.1	25.3±0.1	25.9±0.4	24.8±0.1	24.9±0.1
	Inner leaves	21.9±0.8	23.3±0.1	23.3±0.3	25.0±0.1	25.3±0.1	25.3±0.1	25.9±0.3	24.8±0.1	24.9±0.1

Values are means ± SD (n = 12). Asterisk indicates significant difference from the control according to one-way ANOVA followed by Dunnett's post-hoc test (* = p < 0.05, ** = p < 0.01, *** = p < 0.001)



Fig. S 4 Photos of lettuce plants from the control and treated groups

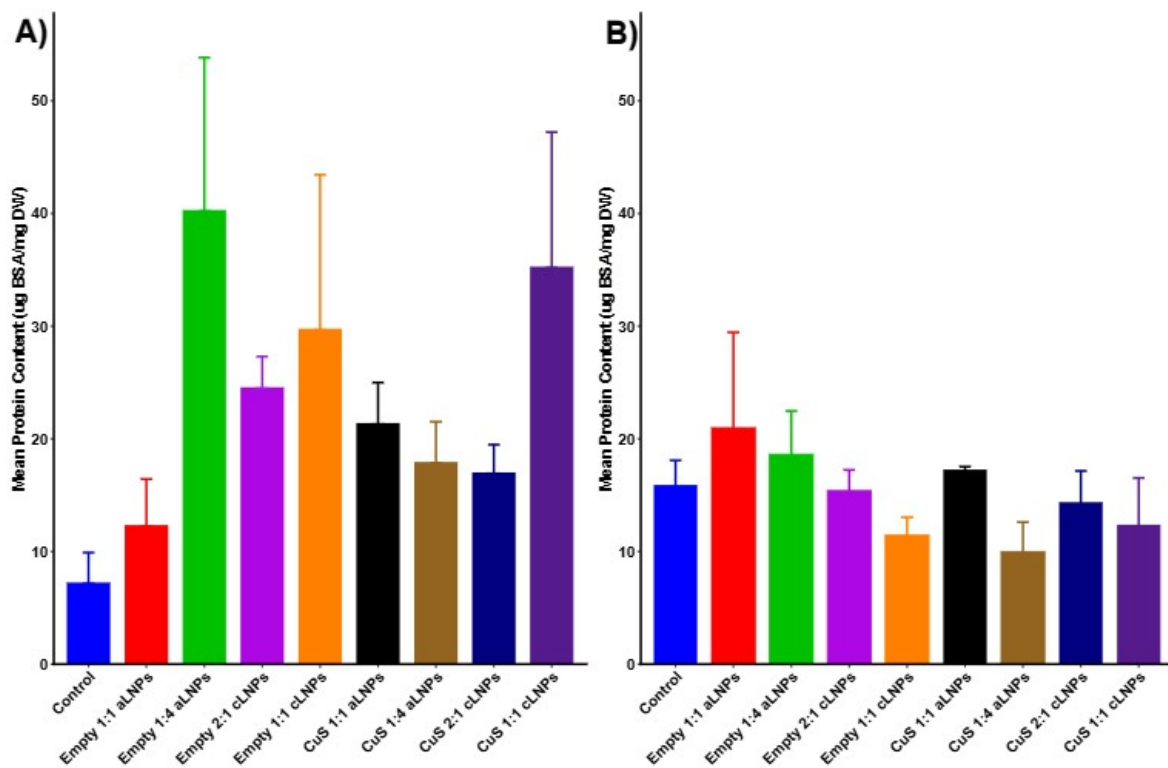


Fig. S 5 Bar charts depicting mean protein content based on a Bradford Protein Assay. Protein content was measured by comparison to a bovine serum albumin calibration curve and colorimetric analysis measured at 562 nm. Bars above plots indicate standard error. A) Mean protein content for leaf tissue. B) Mean protein content for root tissue. No significant differences between the controls and nanoparticles were detected based on ANOVA and Tukey's Honest Significant Differences Test at a significance threshold of $p < 0.05$.