Polymer-Conjugated Carbon Nanotubes for Biomolecule Loading

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Table S1. Polymer characteristics.

Name	Abbreviation	Molecular weight, M_w [g/mol][1]	Polydispersity index[2]
Branched polyethylenimine (800)	BPEI-800	800	0.212 ± 0.043
Linear polyethylenimine (5000)	LPEI-5000	5000	0.563 ± 0.116
Branched polyethylenimine (750k)	BPEI-750k	750,000	0.352 ± 0.044
Branched polyethylenimine (25k)	BPEI-25k	25,000	0.379 ± 0.048
Branched polylysine	-	3,500	0.778 ± 0.125
Linear polyethylenimine (800)	LPEI-800	800	0.633 ± 0.139
Low hydrophobic modified branched polyethylenimine	low-phi-BPEI	25,000 - 30,000	0.131 ± 0.017
Medium hydrophobic modified branched polyethylenimine	med-phi-BPEI	1,500 - 2,000	0.448 ± 0.154

[1] Values reported by manufacturer.

[2] Polydispersity index measured by dynamic light scattering of polymers dissolved at 5 mg mL^{-1} in water, pH 7-8, with a refractive index of 1.529. Values represent the average and standard deviation of three measurements.

Table S2. Peak deconvolution of XPS spectra of COOH-SWNT. Unit = % Peak Area.

Peak	Pristine	Commercial	Commercial, base-washed	In-house	In-house, base-washed
sp^2 C	71.37	57.01	64.09	51.21	50.34
C-OH	10.80	23.16	14.52	23.99	22.60
C=O	7.61	9.17	8.07	11.91	10.33
O=C-OH	3.92	7.06	8.3	10.48	12.57
Sat.	6.30	3.61	5.02	2.40	4.16

Table S3. Size of nanomaterial constructs, characterized by dynamic light scattering (DLS).

Functionalized nanomaterial	Size (d.nm)[1]
COOH-SWNT (Commercial)	147.9 ± 1.484
COOH-SWNT (in-house)	237.0 ± 4.868
PEI-SWNT	160.8 ± 3.037
PEI-SWNT-DNA	130.3 ± 1.670
Trz-H SWNT	300.3 ± 5.848
Trz-PEI SWNT	394.0 ± 3.051
Trz-PEI-SWNT-DNA	779.8 ± 25.934

[1] Values are calculated using the z-average and represent the average and standard deviation of three measurements, with a refractive index of 2.500. Samples are suspended at a pH between 7-8.

Table S4. Elemental analysis of nanomaterial constructs and polymer components.[1]

Material	% C	% H	% N
BPEI-25K	2.02	5.52	1
BPEI-25K-SWNT	2.23	5.81	1

After accounting for sample impurities, we observe that the majority of the signal we obtain can be attributed to BPEI-25K. Assuming a molecular formula of C_2H_5N and a molecular weight of 25000 Mw, we can calculate a ratio of 166 SWNT carbon atoms per BPEI molecule. This corresponds to a polymer:SWNT mass ratio of 12.5:1. Using an estimated SWNT surface area of 1315 m^2 g^{-1}, this translates to a surface density of 9.5 mg m^{-2}.

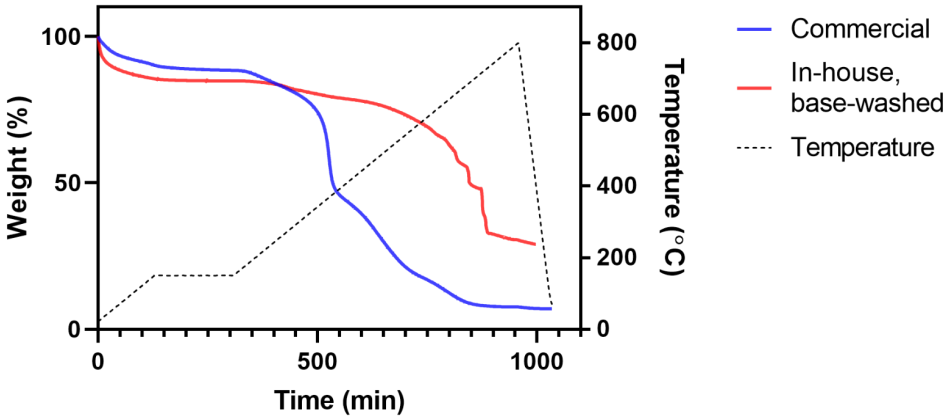

Figure S1. Thermogravimetric analysis heating profile. TGA temperature was increased from room temperature to 150 ˚C, held for 3 hours, then gradually raised to 800 ˚C before being rapidly cooled.

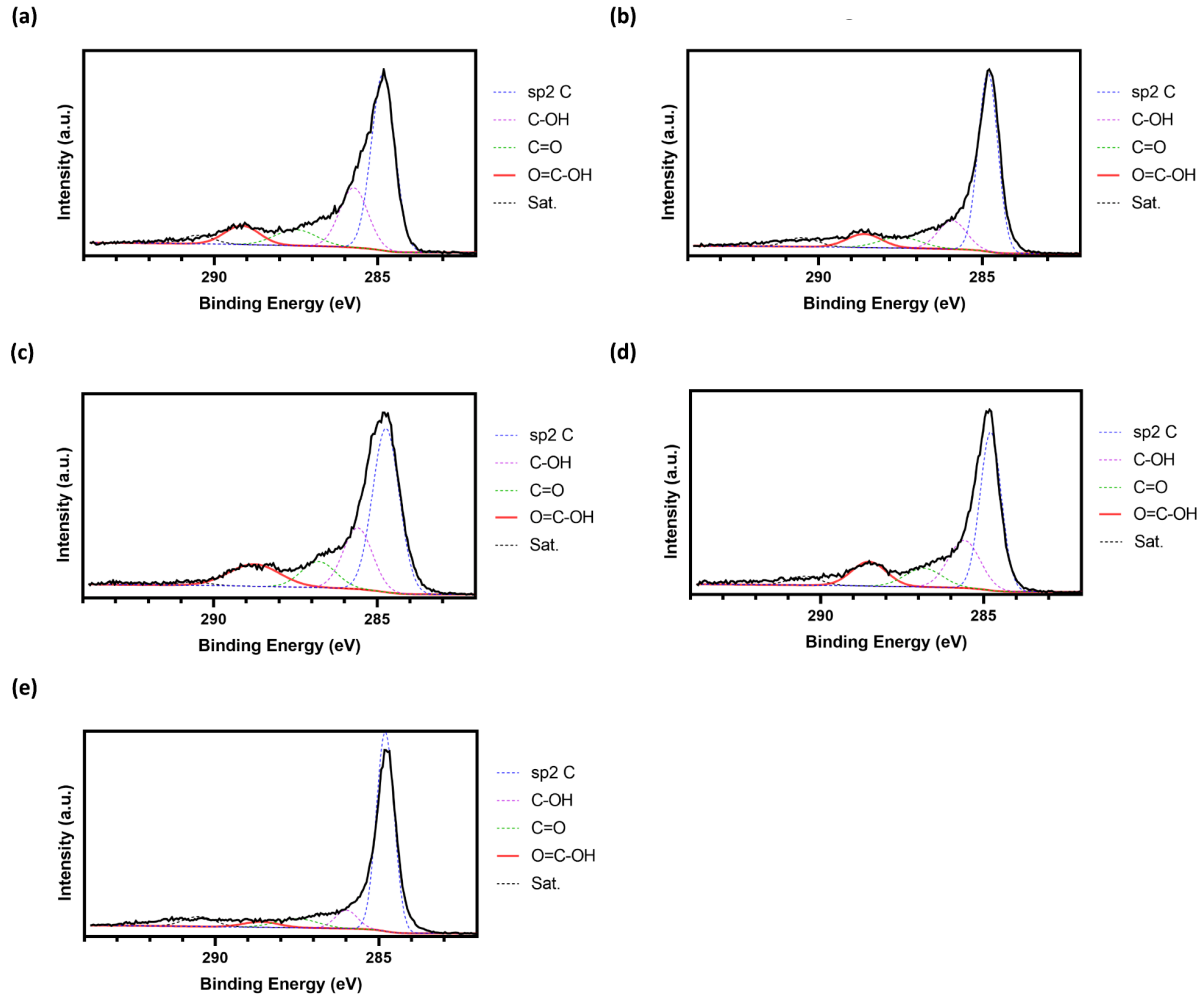

Figure S2. XPS of carboxylated-SWNT preparations. (a) XPS C1s spectra of commercially purchased COOH-SWNT. (b) XPS C1s spectra of commercially purchased COOH-SWNT after wash treatment with 1.0 M NaOH. (c) XPS C1s spectra of in-house carboxylated COOH-SWNT. (d) XPS C1s spectra of in-house carboxylated COOH-SWNT after washing with 1.0 M NaOH. (e) XPS C1s spectra of pristine SWNT.

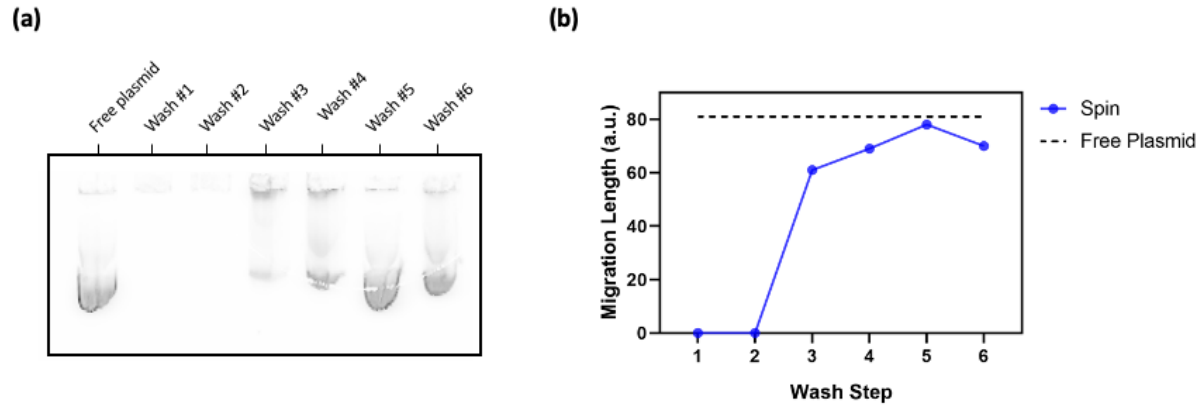

Figure S3. **Washing of BPEI-25k polymer.** Free BPEI-25k polymer suspended in water at 8 mg/L was washed six times with water through a 100 kDa spin filter at 1000 x g. The filtrate was collected after each wash step, loaded with DNA, and run on an agarose gel. By the fourth wash step, the filtrate loaded with DNA ran equidistant to free plasmid, suggesting that no free polymer remains in solution.

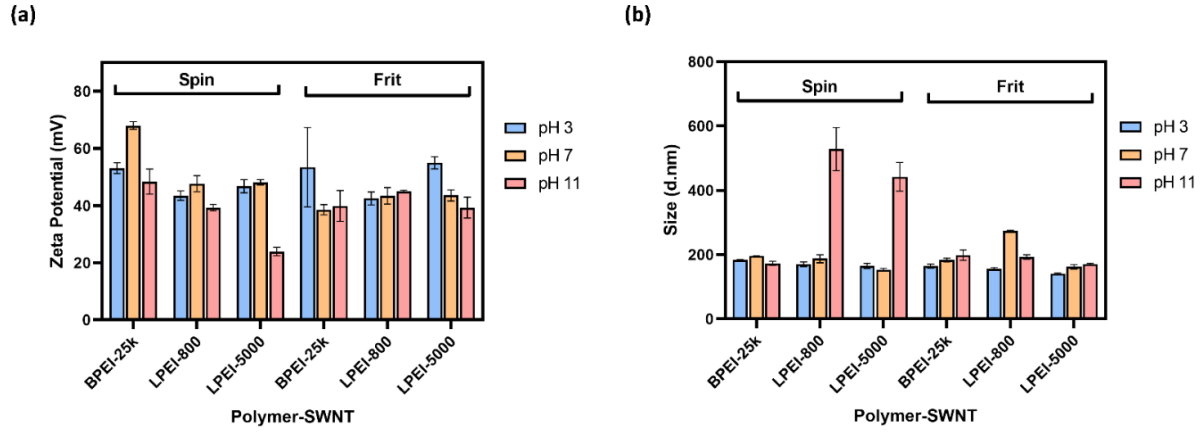

Figure S4. **Zeta potential of polymer-SWNTs washed at varying pH.** We attribute a lower zeta potential value to the poor removal of free polymer. (a) Zeta potential of washed polymer-SWNTs. (b) DLS size measurements for washed polymer-SWNTs. Error bars represent standard deviation of the mean (N = 3).

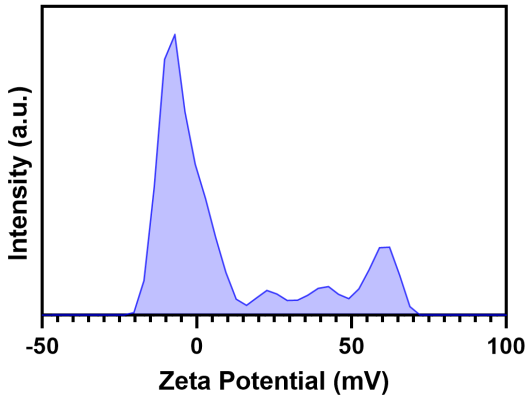

Figure S5. Zeta potential of BPEI-25k polymer in water. Free polymer suspended in solution exhibits a neutral or weakly positive charge, likely due to intermolecular interactions. When free polymer is suspended with positively charged polymer-SWNT nanoparticles, this can increase the ionic strength of the suspension and lower the measured zeta potential of the whole solution.

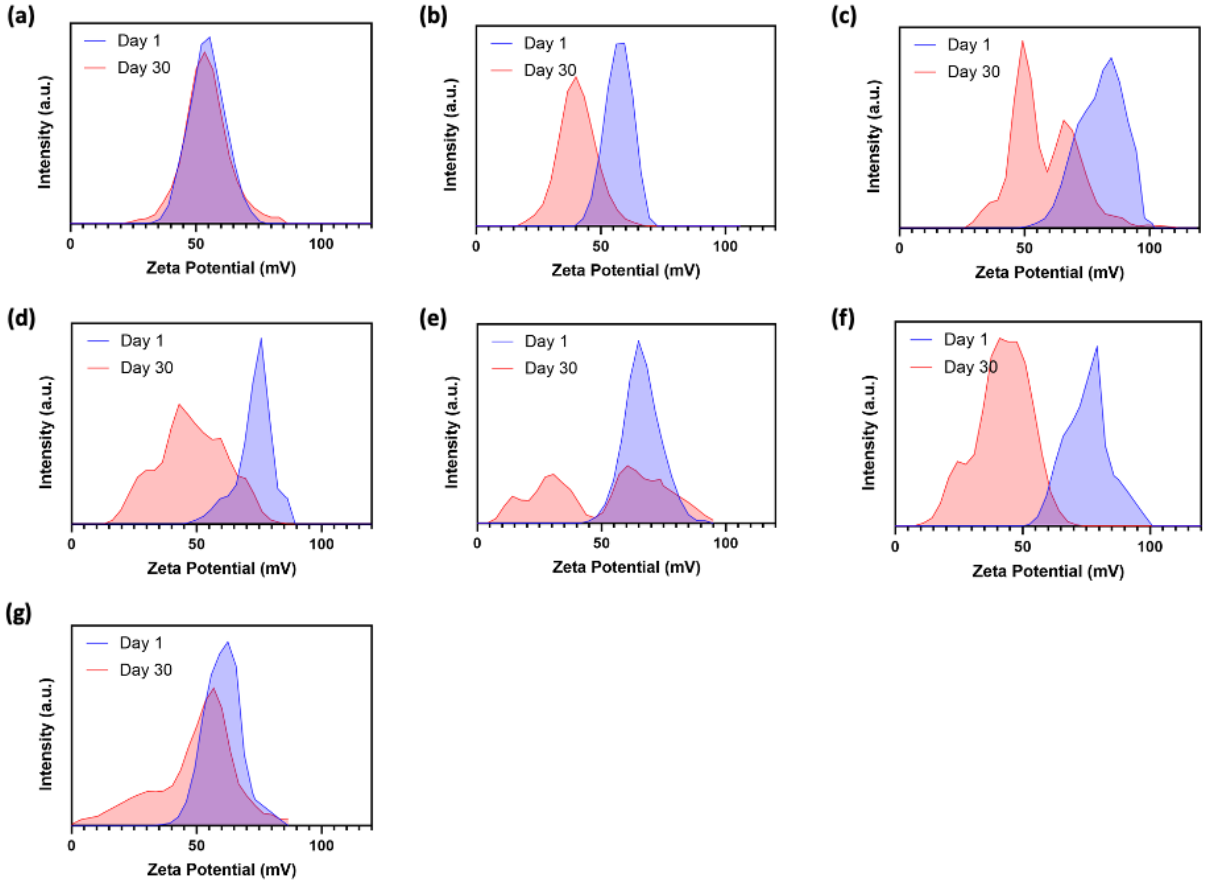

Figure S6. **Desorption of polymer from polymer-SWNT nanoparticles over time.** (a) BPEI-800, (b) LPEI-5000, (c) BPEI-750k, (d) Branched polylysine, (e) LPEI-800, (f) low-phi-BPEI, (g) med-phi-BPEI.

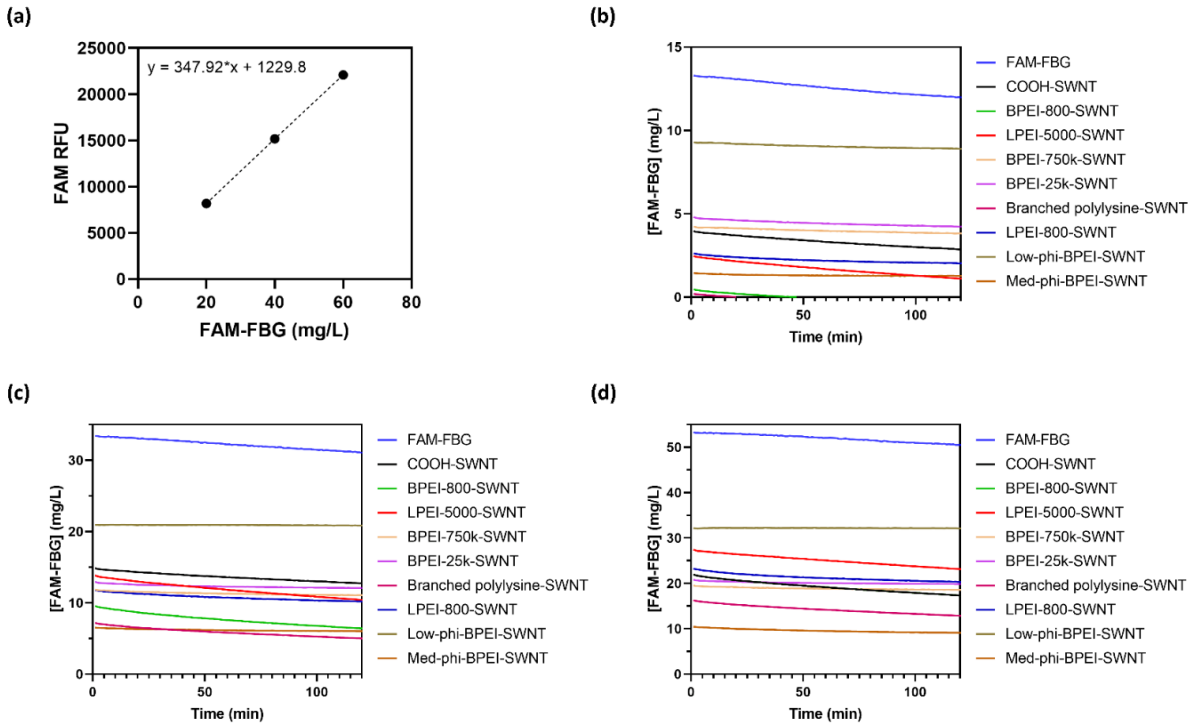

Figure S7. Quantification of real-time FAM-fibrinogen adsorption to polymer-SWNTs. (a) FAM-FBG fluorescence calibration curve. (b) Adsorption of 20 µg/mL FAM-FBG determined by quenching of conjugated FAM fluorophore to 5 µg/mL polymer-SWNTs. (c) Adsorption of 40 µg/mL FAM-FBG determined by quenching of conjugated FAM fluorophore to 5 µg/mL polymer-SWNTs. (d) Adsorption of 60 µg/mL FAM-FBG determined by quenching of conjugated FAM fluorophore to 5 µg/mL polymer-SWNTs. The decrease in the FAM-FBG concentration over time across all concentrations can be attributed to self-quenching.

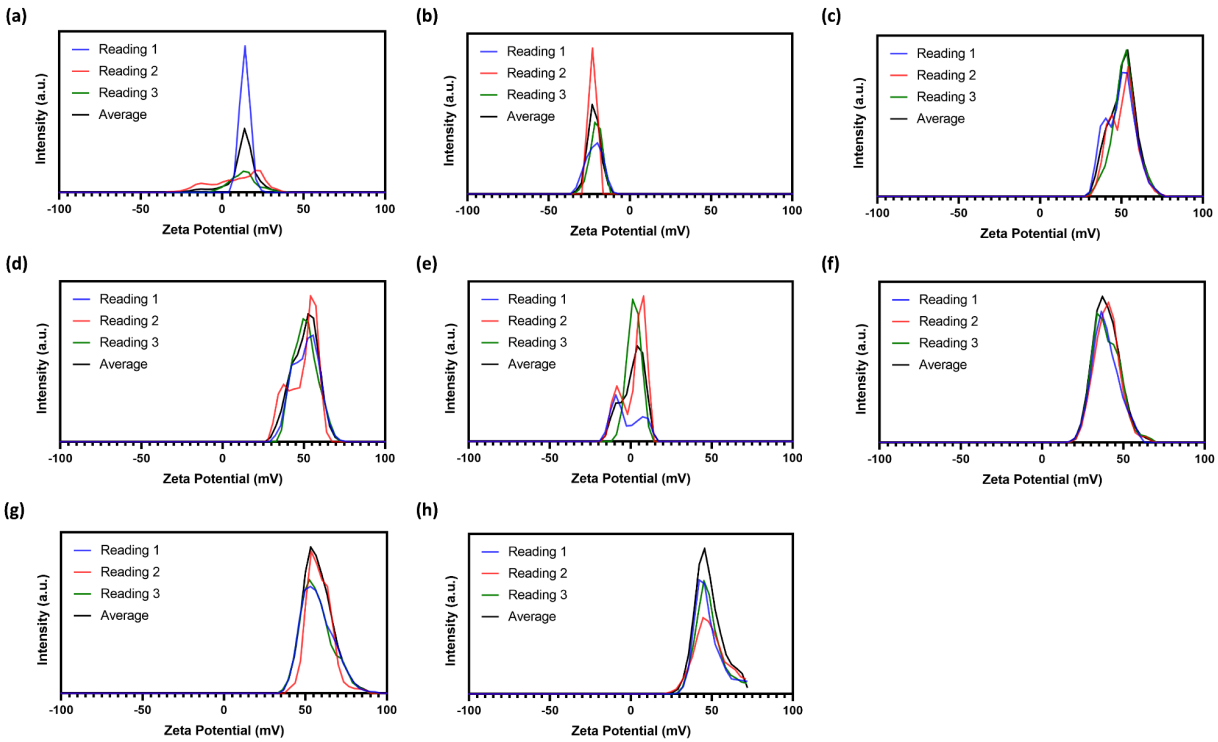

Figure S8. Zeta potential measurements of DNA-polymer-SWNTs. (a) BPEI-800, (b) LPEI-5000, (c) BPEI-750k, (d) BPEI-25k, (e) Branched polylysine, (f) LPEI-800, (g) low-phi-BPEI, (h) med-phi-BPEI.

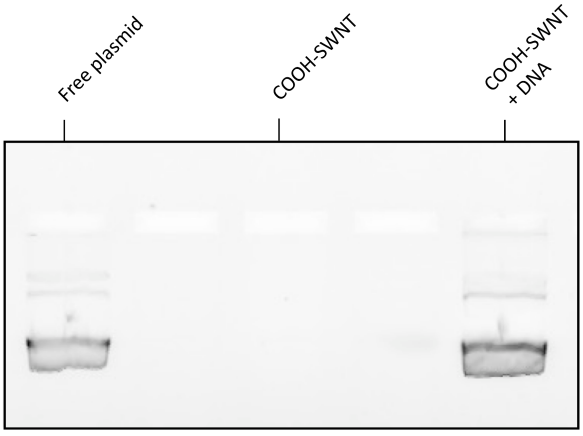

Figure S9. DNA does not adsorb to COOH-SWNTs. COOH-SWNT (100 ng) was added to DNA (100 ng) and diluted to a total volume of 10 µL. The sample was loaded with 6x non-SDS containing loading dye and run in 0.8% agarose at 80 V for 45 min. We observe a prominent band equivalent to the free plasmid control, indicating that the DNA does not adsorb to COOH-SWNT, in contrast to most SWNTs that have been functionalized with cationic polymers that do bind DNA (Figure 5c).

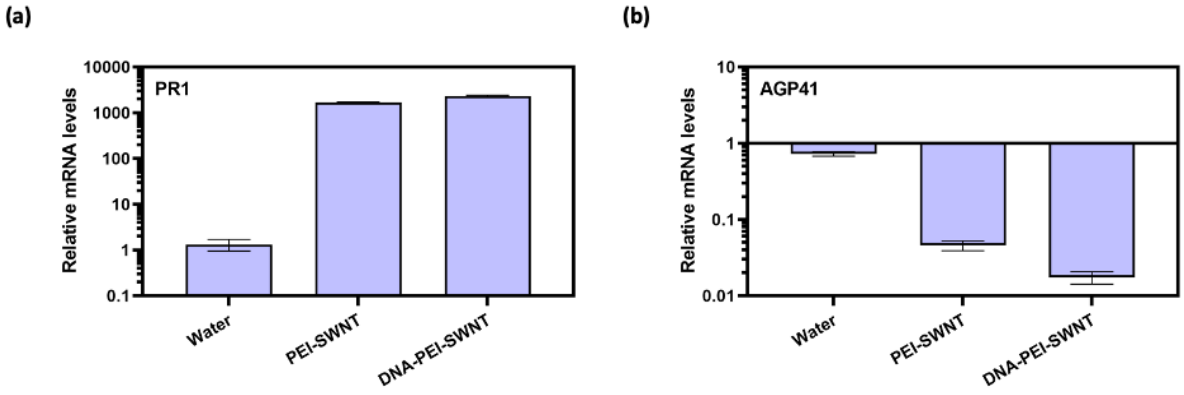

Figure S10. Toxicity impact of DNA loading on polymer-SWNTs. We observe no notable difference in trends of mRNA fold change of *arabinogalactan protein 41* (AGP41) between *Arabidopsis thaliana* leaves infiltrated with BPEI-25k-SWNT and DNA-BPEI-25k-SWNT two days post-infiltration. Error bars represent standard error of the mean (N = 3).

References

1. Gonzalez-Grandio, E., Demirer, G.S., Jackson, C., Yang, D., Landry, M.P. Carbon Nanotube Biocompatibility in Plants Is Determined by Their Surface Chemistry. 2021. *J Nanobiotechnol*. In Press.