Supporting Information for:

Polymer Cloaking Modulates the Carbon Nanotube Protein Corona and Delivery into Cancer Cells

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Supporting Figures

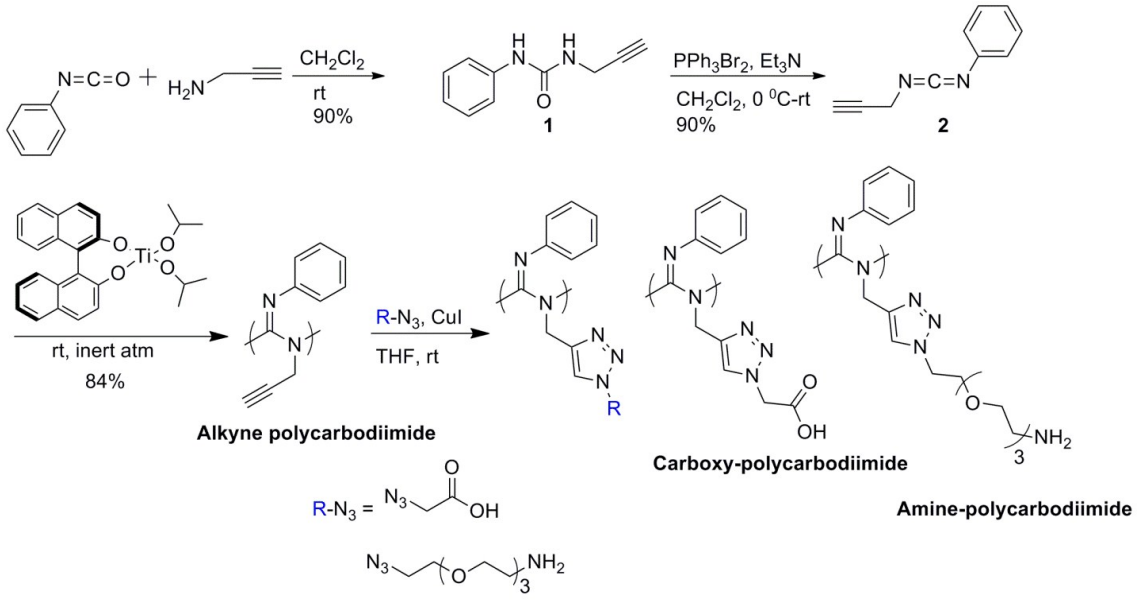

Figure S1. Synthesis of polycarbodiimide polymers.

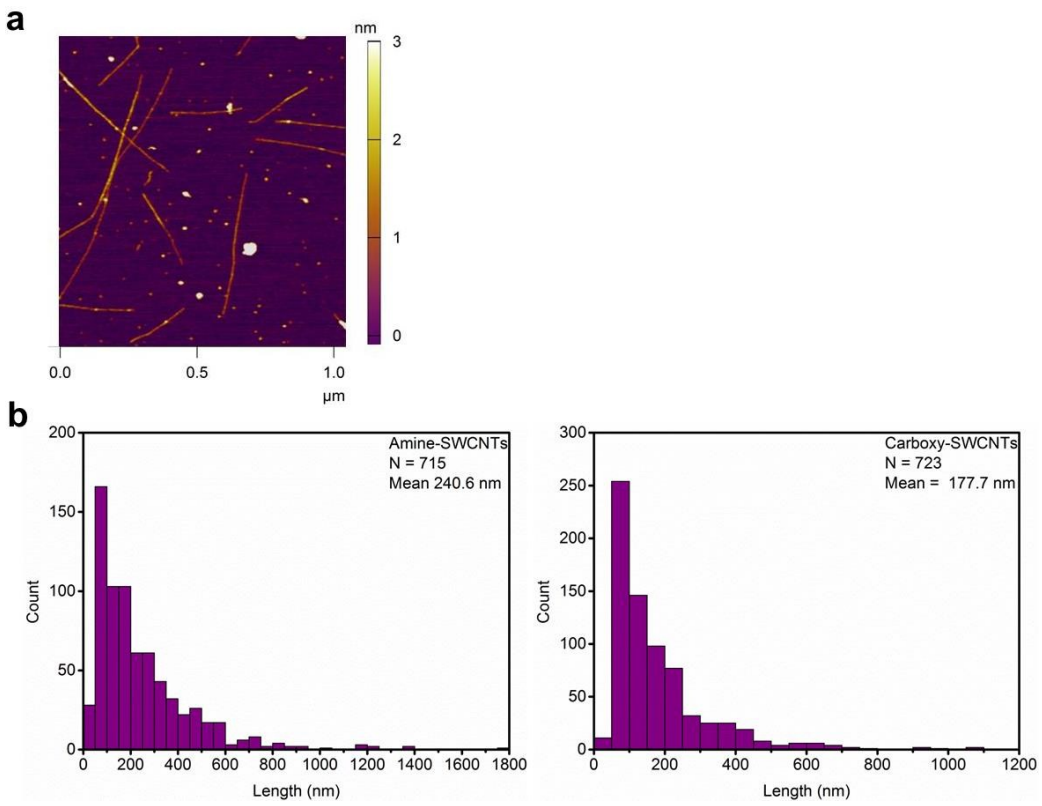

Figure S2. Atomic force microscopy (AFM) of polymer-SWCNTs. **a**) Representative AFM micrograph of Amine-SWCNTs. **b**) Length distribution of the samples as quantified from AFM images of amine-SWCNTs (left) and carboxy-SWCNTs (right).

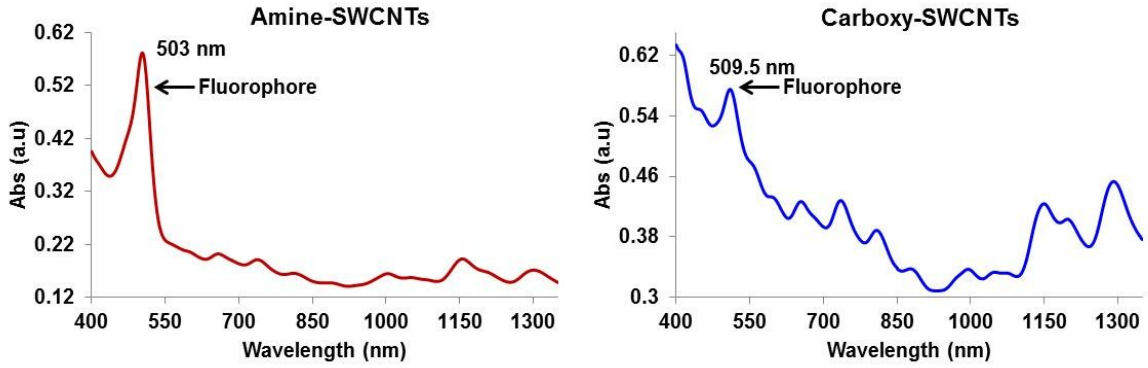

Figure S3. UV-Vis-near-infrared (NIR) absorption spectra of fluorophore -polymer-SWCNT complexes in water, showing peaks from fluorophore (fluorescein was conjugated to amine-SWCNTs and Alexa 488 was conjugated to carboxy-SWCNTs) and SWCNTs.

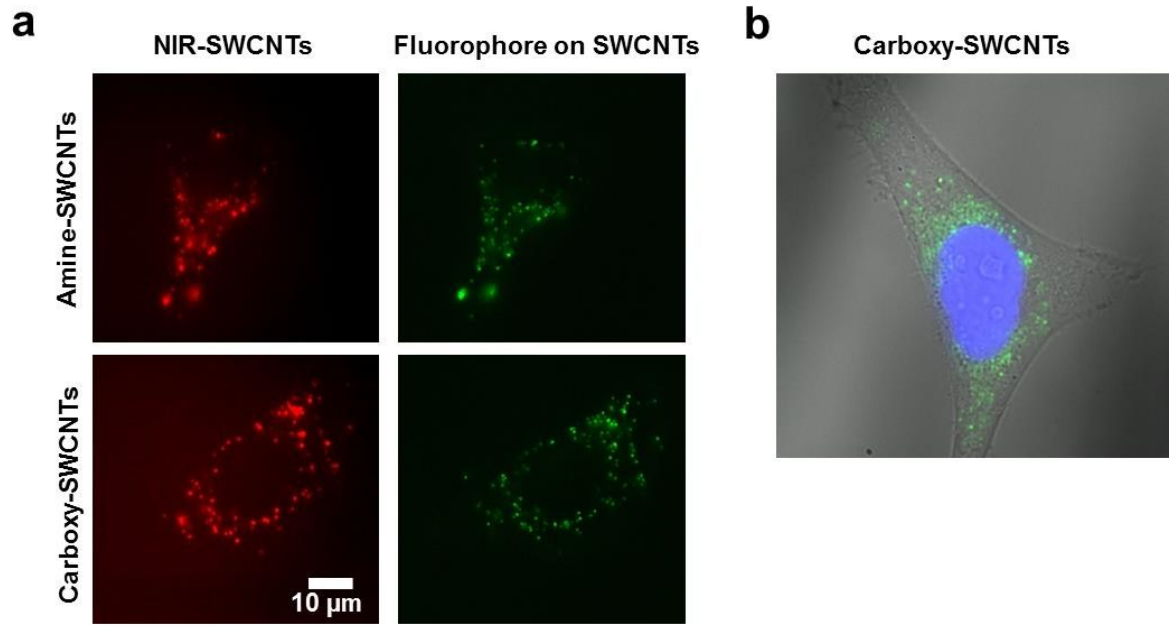

Figure S4. a) Nanotube visualization in cells using two emitters. NIR photoluminescence (left) and visible fluorescence (right). HeLa cells were incubated in the presence of polymer-carbon nanotube complexes either in normal media (amine-SWCNTs) or low-serum media (carboxy-SWCNTs). The images were collected using a microscope equipped with CCD camera (QIClick, QImaging) and 2D InGaAs array (Photon etc.). The visible fluorescence images were collected using an X-cite 120Q lamp (Lumen Dynamics) with FITC filter set and conventional CCD camera (QIClick, QImaging) while near-infrared images were collected using a 2D InGaAs array (Photon etc.). Overlays were not shown due to different pixel sizes and aspect ratios of two different cameras used in the experiments. **b)** HeLa cell after incubation with carboxy-SWCNTs and staining with nuclear dye. Fluorophore on SWCNTs is shown in green. Hoechst nuclear stain is shown in blue. Images were processed using ImageJ software (NIH). All images are at the same scale (scale bar 10 µm).

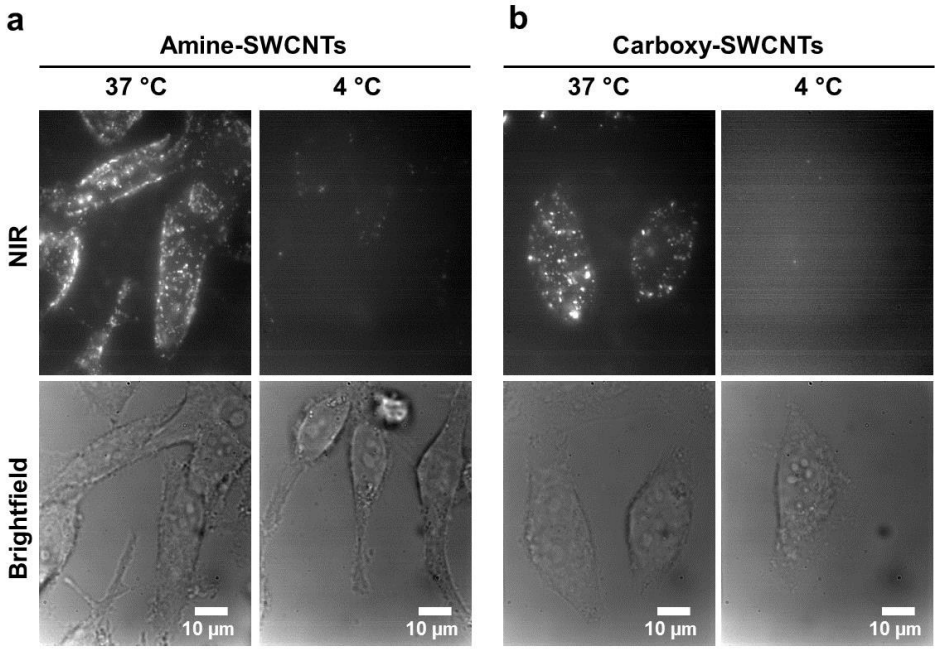

Figure S5. Carbon nanotube uptake in cells at different temperatures. Cells were incubated with **a**) amine-SWCNTs and **b**) carboxy-SWCNTs.

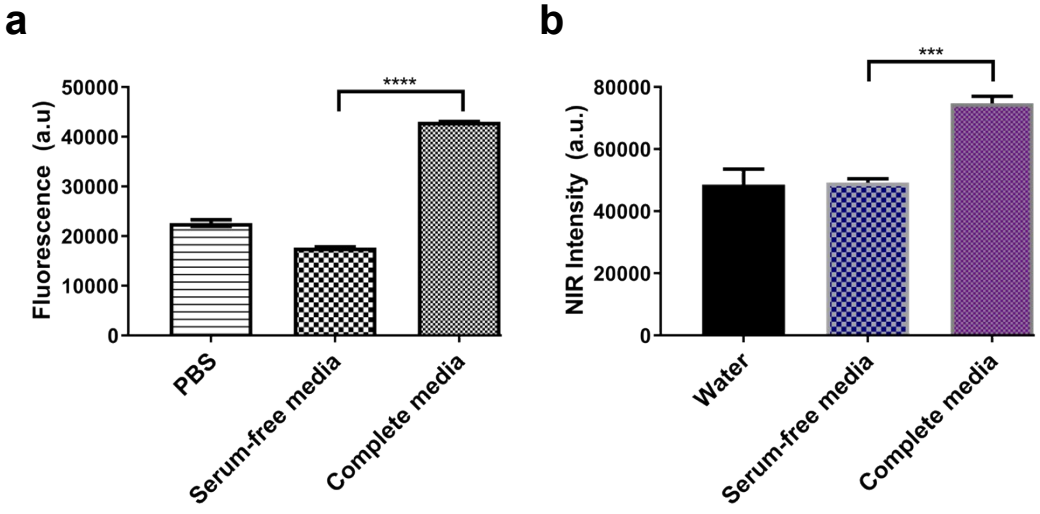

Figure S6. Effect of cell culture media on carboxy-SWCNTs emission. **a**) Visible fluorescence from Alexa 488/fluorescein on the polymer-nanotube complexes. **b**) NIR fluorescence of carbon nanotubes on the complexes (concentration = 0.8 mg/L). A phenol red-free media was used in panel a (nanotube concentration = 2 mg/L).

Supporting Methods

Chemicals

Chemicals were purchased from Sigma-Aldrich, Milwaukee, WI, Acros Organics, and Fisher Scientific, Fair Lawn, NJ, and used as received. Neutral silica gel (Ultrapure 60–200 µm, 60 Å, Acros Organics) was

used for column chromatography purification of monomers. Anhydrous and inhibitor-free tetrahydrofuran (THF) was used for click chemistry.

Polymer synthesis and characterization
Small molecules and polymers were synthesized and characterized according to literature procedure[1].
Alkyne polycarbodiimide polymer. FTIR (thin film, cm^{-1}): characteristic absorption from terminal alkyne group and polymer backbone; 3304 (terminal alkyne C–H), 2123 (alkyne triple bond, C≡C), 1631 (polymer backbone, C=N). Mn = 36, 608, PDI = 1.35. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): reference TMS = 0 ppm, δ = 7.28–6.84 (br), 5.35–5.29 (br), 4.37–4.20 (br), 3.14 (br), 2.07–0.75 (br). 11-Azido-3,6,9-trioxaundecan-1-amine and Azide-fluor-488 were used as received. The azides were coupled to the alkyne polymer by Cu(I) catalyzed azide-alkyne cycloaddition 'click' chemistry following a literature procedure[2]. Manipulations were performed under inert atmosphere. Briefly, alkyne polymer (20 mg) dissolved in tetrahydrofuran (THF, 5 mL, anhydrous, inhibitor free) at room temperature was mixed with azide solution (in THF, 1mL) at 1:1.5 mol equivalents per alkyne unit in the polymer. To label the polymers with fluorophore, a mixture of azides consisting of either azido acetic acid and azide-fluor-488 (1: 0.008 mol equivalents) or a mixture of 11-Azido-3,6,9-trioxaundecan-1-amine and FITC-PEG_3-azide (1:0.03mol equivalents) was added to the reaction mixture. The reaction mixture was stirred for five minutes followed by addition of CuI (3.0 equiv) and triethyl amine (6.0 equiv). The reaction mixture was stirred overnight (12 h) and the completion of the coupling reaction was confirmed by FTIR analysis of the resulting polymers. The products were washed with THF (10 mL, 3X) to remove unreacted small molecules, dried and characterized by FITR spectroscopy. Limited solubility of the final polymers posed difficulty in GPC analysis and NMR spectroscopies.
Amine polycarbodiimide polymer **FTIR** (NaCl thin film, 4000 cm^{-1} – 800 cm^{-1}): 3426.8, 3007.4, 2992.0, 2919.8, 1657. 6, 1627, 1437.5, 1439.8, 1277.6 (from solvent), 1262.7 (from solvent), 1026.7, and 954.9.
Carboxy polycarbodiimide polymer **FTIR** (NaCl thin film, 4000 cm^{-1} – 800 cm^{-1}): 3620.7, 3151.9, 2663.8, 2849.1, 1737.0, 1675.3, 1586.6, 1488.0, 1368, 1182.7, 1073, and 972.0.

Polymer-nanotube complex characterization
The polymer-SWCNT aqueous suspensions were prepared as described in the main text. The visible-near-infrared (VIS−NIR) absorption spectra were measured with a JASCO V-670 spectrophotometer. Near infrared photoluminescence excitation/emission (PL) measurements were performed on a home-built instrument consisting of an IsoPlane SCT 320 spectrograph and PioNIR InGaAs detector (Princeton Instruments) connected to an Olympus IX71 inverted microscope. A 20× objective was used. Samples were excited using a SuperK Extreme supercontinuum laser connected to a Varia variable bandpass filter (NKT Photonics). The excitation wavelength was varied from 491 to 824 nm, and the emission was recorded from 915 to 1354 nm. Data were collected using a custom Labview (National Instruments) automation program. Spectral corrections for wavelength-dependent excitation power, non-linearity in the InGaAs detector response and background subtraction were applied to data. These data were then analyzed and plotted using Matlab (The MathWorks) code.

Zeta potential (surface charge) measurements were conducted at room temperature using a Zetasizer Nano-ZS instrument (Malvern). The measurements were performed on the polymer-nanotube complexes (1-2 mg/L) in ultrapure water (18.2 mΩ) in a 1 mL folded capillary cell (Malvern). The ζ-potential values are within the range reported for surfactant-suspended SWCNTs[3]. Surface potential on the polymer-SWCNTs were also measured after treating with complete cell culture media supplemented with FBS (10%). The polymer-SWCNTs were mixed with cell culture media (1mL) for 30 minutes at room temperature. The mixture was centrifuged (30 000 rcf) for 10 minutes and the supernatant was removed to decrease the ionic strength of the solution. The pellete was washed and suspended in water with brief sonication (10 sec) and ζ-potential was measured.

Morphology characterization by atomic force microscopy was performed at room temperature using Asylum MFD-3D-BIO (Asylum Research) in AC mode. The polymer-nanotube complexes (~1 mg/L) were deposited onto a freshly cleaved mica surface (Pelco Mica Disc, V1, Ted Pella), washed and dried at room temperature with ultrapure nitrogen prior to imaging. The scan size was 2 μm X 2 μm and scan rate was 0.25 Hz–0.5 Hz. The images were processed with Igor software.

Cell viability tests were performed using the Tali Viability Kit - Dead Cell Red (Invitrogen) in a Tali Image-Based Cytometer following the manufacturer's protocol. Cells were plated in a 24-well clear bottom dishes 24 h prior to the experiments. Cells at 70-80% confluence were incubated with 0.1 mg/L to 1 mg/L polymer-nanotube complexes for 24–48 h at 37 °C in humidified air with 5% CO_2. Cells incubated under the same conditions but without nanotubes were used as the control. The experiments were performed in triplicate and the results were compared to control cells.

Energy-dependent uptake
Cells were plated in a 24-well clear bottom dishes used at 70-80% confluence. Cells were incubated with either amine-SWCNTs (0.2 mg/L) in the presence of complete cell culture media supplemented with 10% FBS or carboxy-SWCNTs (0.5 mg/L) in the presence of low serum media (0.2 % FBS) for 60 minutes at 37 °C or 4 °C. A low serum condition was employed for carboxy-SWCNTs uptake experiments to facilitate the uptake of nanotubes. A high serum conditions (10% FBS) was found to significantly inhibit uptake of nanotubes posing a difficulty in analysis.

References

1. J. Budhathoki-Uprety, P. V. Jena, D. Roxbury and D. A. Heller, *J Am Chem Soc*, 2014, **136**, 15545-15550.
2. J. Budhathoki-Uprety and B. M. Novak, *Macromolecules*, 2011, **44**, 5947-5954.
3. B. White, S. Banerjee, S. O'Brien, N. J. Turro and I. P. Herman, *J Phys Chem C*, 2007, **111**, 13684-13690.