Supporting Information

Controlling the Length of Self-Assembled Nanotubes by Sonication Followed by Polymer Wrapping

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Experimental Section

General Methods. Atomic force microscopy (AFM) was conducted in tapping mode under a nitrogen atmosphere. Transmission Electron Microscopy (TEM) was carried out with Technai G2 Spirit instrument operating at 80 kV. ^{1}H and ^{13}C NMR were recorded at 400 MHz on a Bruker Avance III instrument. ESI mass spectra were recorded on a Bruker MicrOTOF coupled with HPLC. MALDI-TOF mass spectra were done on a Bruker MicroFlex (MALDI) mass spectrometer equipped with a pulsed UV 335 nm nitrogen laser. Spectra were acquired in positive ion and linear modes with an acceleration voltage of 20 kV. The sonications were carried out using a Fisher Scientific 100 ultrasonic processor equipped with a 3.2 × 15 cm converter and a 3 mm diameter probe. The ultrasonic processor was operated at 22.5 kHz and at 10-60% of maximum amplitude. All UV-Vis spectra were recorded with a SHIMADZU UV-2450 at 25 ^{o}C. All fluorescence spectroscopy were performed in a SHIMADZU RF-5301 using a cuvette with 3 mm pass length at 25 ^{o}C. Circular dichiroism (CD) spectra were recorded using a AVIV 202 CD spectrometer at 25 ^{o}C. All reactions were performed under a nitrogen atmosphere. Dimethylformamide (DMF) was dried by distillation from $MgSO_4$; dichloromethane was distilled from calcium hydride; chloroform was distilled form calcium carbonate. Chromatographic separations were performed on silica gel 60 (230-400 mesh, 60 Å) using indicated solvents. Poly[2,6-(4,4-bis-sodium butanylsulfonate-4H-cyclopenta-[2,1-b;3,4-b']-dithiophene)*alt*-1,4-phenylene] (PCT-SO_3Na) was obtained from One-Material, Canada. The molecular weight of PCT-SO_3Na was determined by GPC (DMF): M_W = 7800, DP = 13, PDI = 1.08.[1] Commercial curdlan (Wako Pure Chemical Industries, Ltd.) was used to prepare curdlan sulfate (Cur-SO_3Na). The content

of sulfate group was measured on a Perkin-Elmer Optima 8300DV inductively coupled plasma optical emission spectrometer (ICP). Piperidine-*N*-sulfonic acid was prepared from piperidine and chlorosulfonic acid according to the method of Nagasawa and Yoshidome.[2]

Sonication of NDI-Bola Nanotubes. NDI-Bola was synthesized according to our previous work.[3] Freeze-dried **NDI-Bola** (5.5 mg) was added to HPLC-grade water (500 μL), and the mixture was sonicated until the solid dissolved. The self-assembly of **NDI-Bola** completed within 12 h. Then, preformed **NDI-Bola** nanotubes were fragmented by sonication with cooling in an ice bath. The ultrasonic processor was operated at 10-60% of maximum amplitude. After continuous sonication for 3 min to circulate **NDI-Bola** solution around the submerged probe effectively, the shortened **NDI-Bola** nanotubes were characterized immediately.

Synthesis of Curdlan Sulfate (Cur-SO_3Na). Curdlan sulfate was synthesized according to literature.[4] Curdlan (0.5 g, 3.1 mmol, calculated as glucose monomer) in anhydrous pyridine (80 mL) was stirred for 2 h at 25 ^oC to afford a transparent solution. To the solution was added chlorosulfonic acid (4.4 g, 33.2 mmol). The mixture was stirred at 100 ^oC for 60 min. With cooling in an ice bath, the reaction mixture was neutralized by the addition of saturated $NaHCO_3$ solution (120 mL) resulting in the formation of a precipitate. The precipitate was collected by centrifugation (5000 rpm, 10 min), washed with acetone (3 times), redissolved in water (50 mL), and dialyzed (molecular weight cut off: 3.5 kDa) against deionized water for 3 d. The dialysate was freeze-dried to give Cur-SO_3Na. The molecular weight of Cur-SO_3Na was determined by MALDI-TOF: M_W =

4600, PDI = 1.06, α-cyano-4-hydroxycinnamic acid as matrix. Anal. Found (ICP): S, 17.0%.

Preparation of Shortened Polymer-Nanotube Composites. After sonication, shortened **NDI-Bola** (20 mM) nanotubes were mixed with polymer solutions under stirring with a Vortex mixer immediately and kept at 20 ^{o}C. After incubation (3 or 7 d), polymer-nanotube composites were diluted with deionized water and centrifuged at 5000 rpm for 15 min. Under these centrifuge conditions, neither the isolated polymer nor the **NDI-Bola** monomer forms a pellet. The resulting supernatants were decanted off, and pure polymer-nanotube composites were collected in pellets and redispersed in water for following microscope measurements. The concentration of **NDI-Bola** in samples for AFM and TEM studies was 2 mM.

Transmission Electron Microscopy (TEM). Diluted pellets of **NDI-Bola**/PCT-SO_3Na and **NDI-Bola**/Cur-SO_3Na after centrifugation (the concentration of **NDI-Bola** was 2 mM) were dropped on carbon-coated copper grids (Ted Pella, Inc.) for 10 min. After removal of excess solution, the sample grid was negatively stained with 2% (w/w) uranyl acetate solution for 2 min. The dried specimen was observed with Technai G2 Spirit TEM instrument operating at 80 keV. For tilted-beam TEM (TB-TEM), nanotubes were adsorbed to carbon films on lacey carbon supports on 200 mesh copper grids. A 5-μL aliquot of nanotube-polymer solutions (the concentration of **NDI-Bola** was 2 mM) and a 1-μL aliquot of TMV solution were applied simultaneously to the grid. After a 5-min adsorption period, excess solution was removed with filter paper. TB-TEM images were acquired at 60,000 × magnification, using a beam tilt angle of 0.55°. Before recording TB-TEM images, the beam was centered and spread to produce uniform

illumination over the field of view. TB-TEM images were analyzed with ImageJ software. Only nanotubes that appeared to be single filaments, rather than pairs or higher-order bundles, were selected for MPL measurements. Square areas of 25 × 25 nm were chosen to allow no less than 20 MPL counts for each sample.

Atomic Force Microscopy (AFM). AFM images were collected on Bruker AXS Dimension Icon Atomic Force Microscope under a nitrogen atmosphere in tapping mode using silicon tips (NSC14/AIBS, μMasch). Diluted pellets of **NDI-Bola**/PCT-SO_3Na were dropped on freshly cleaved mica and allowed to dry for 30 min before imaging. Length distributions were extracted from AFM images. After sonication, diluted **NDI-Bola** (1 mM) was drop-casted on freshly cleaved mica. AFM images (3.0 × 3.0 μm) were taken at a line frequency of 0.5 Hz and at a resolution of 512 × 512 pixels. Obtained AFM images were analyzed with Bruker NanoScope Analysis (version 1.40) software.

MALDI-TOF Mass Spectrometry. MALDI-TOF MS measurements were done on a Bruker MicroFlex (MALDI) mass spectrometer equipped with a pulsed UV 335 nm nitrogen laser. Spectra were acquired in positive ion and linear modes with an acceleration voltage of 20 kV. The data was summed over 100 laser shots. The laser power was tuned to yield sufficient signals. Analyte solutions were prepared by mixing 100 μL of saturated matrix solution (α-cyano-4-hydroxycinnamic acid, HCCA, in acetone) and 100 μL of Cur-SO_3Na (5.0 mg/mL) in water. 1.0 μL of the resulting mixture was spotted on a MALDI sample plate and dried in air.

Circular Dichroism (CD) Spectrometry. CD spectra were recorded on a AVIV 202 CD spectrometer equipped with a Peltier temperature controller under a nitrogen atmosphere. Spectra were collected at 25 ^{o}C in a quartz cell with 1 mm path length over a wavelength

range of 190-650 nm with a step size of 0.5 nm. All scans were conducted at continuous mode at the scanning speed of 100 nm/min. Each CD spectrum was averaged from 3 scans with an integration time of 2 s.

Fluorescence Spectroscopy Measurements. Aqueous samples for steady state fluorescence study (the concentration of **NDI-Bola** in all samples was 2 mM) were prepared by dilution form 20 mM **NDI-Bola** or pellets of **NDI-Bola**/PCT-SO_3Na after centrifuge (5000 rpm). All samples were equilibrated for 24 h before measurements. Fluorescence emission spectra of NDI were measured with excitation at 330 nm.

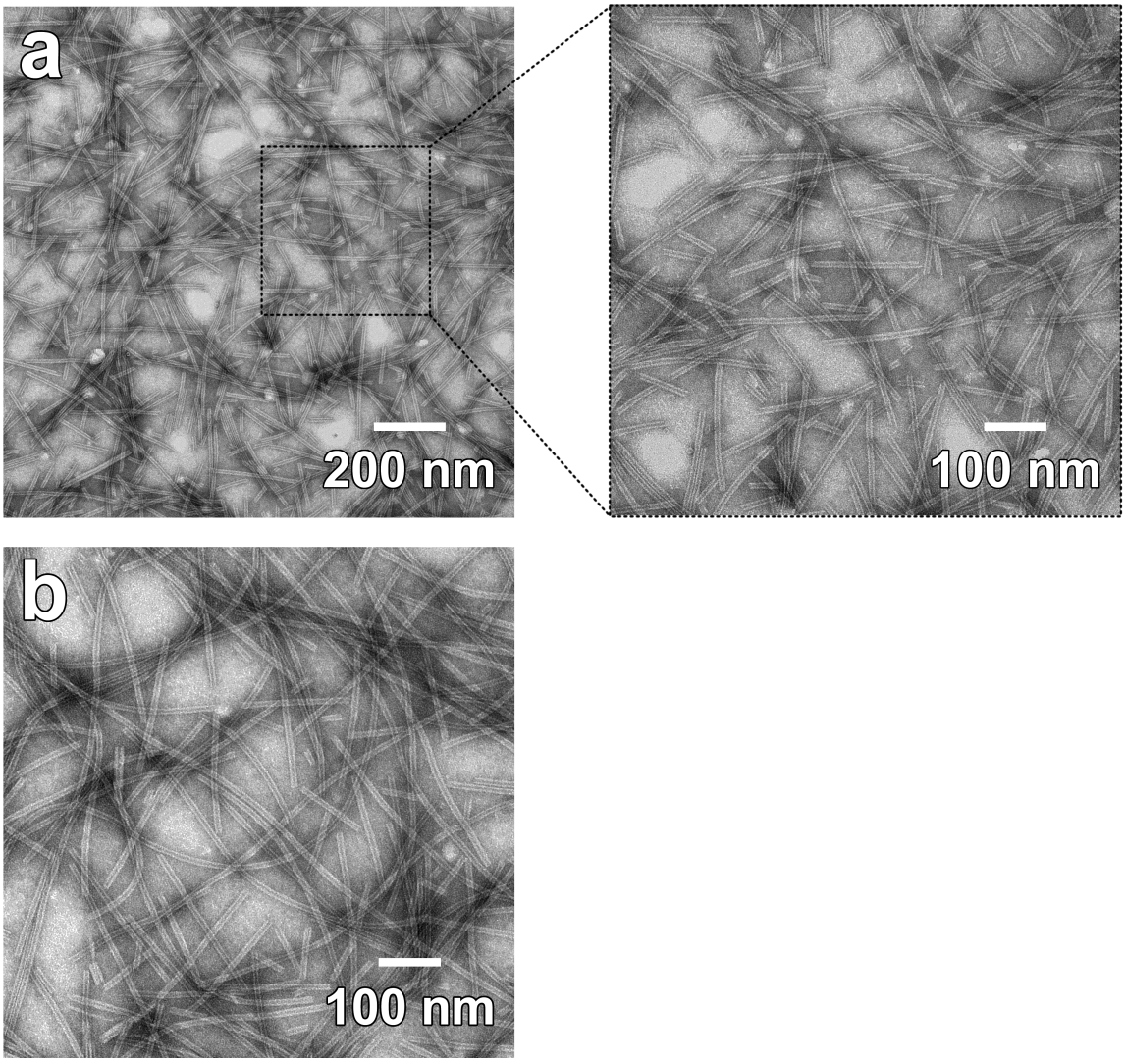

Figure S1. Preformed **NDI-Bola** (20 mM) nanotubes were sonicated at 10% amplitude for 3 min. (a) TEM images of shortened **NDI-Bola** (2 mM) in water diluted from 20 mM solution after sonication. As a control, the short **NDI-Bola** fragments (20 mM) were aged for 72 h to allow structure recovery at ambient temperature. (b) TEM image of recovered **NDI-Bola** (2 mM) in water. 2% (w/w) uranyl acetate as the negative stain.

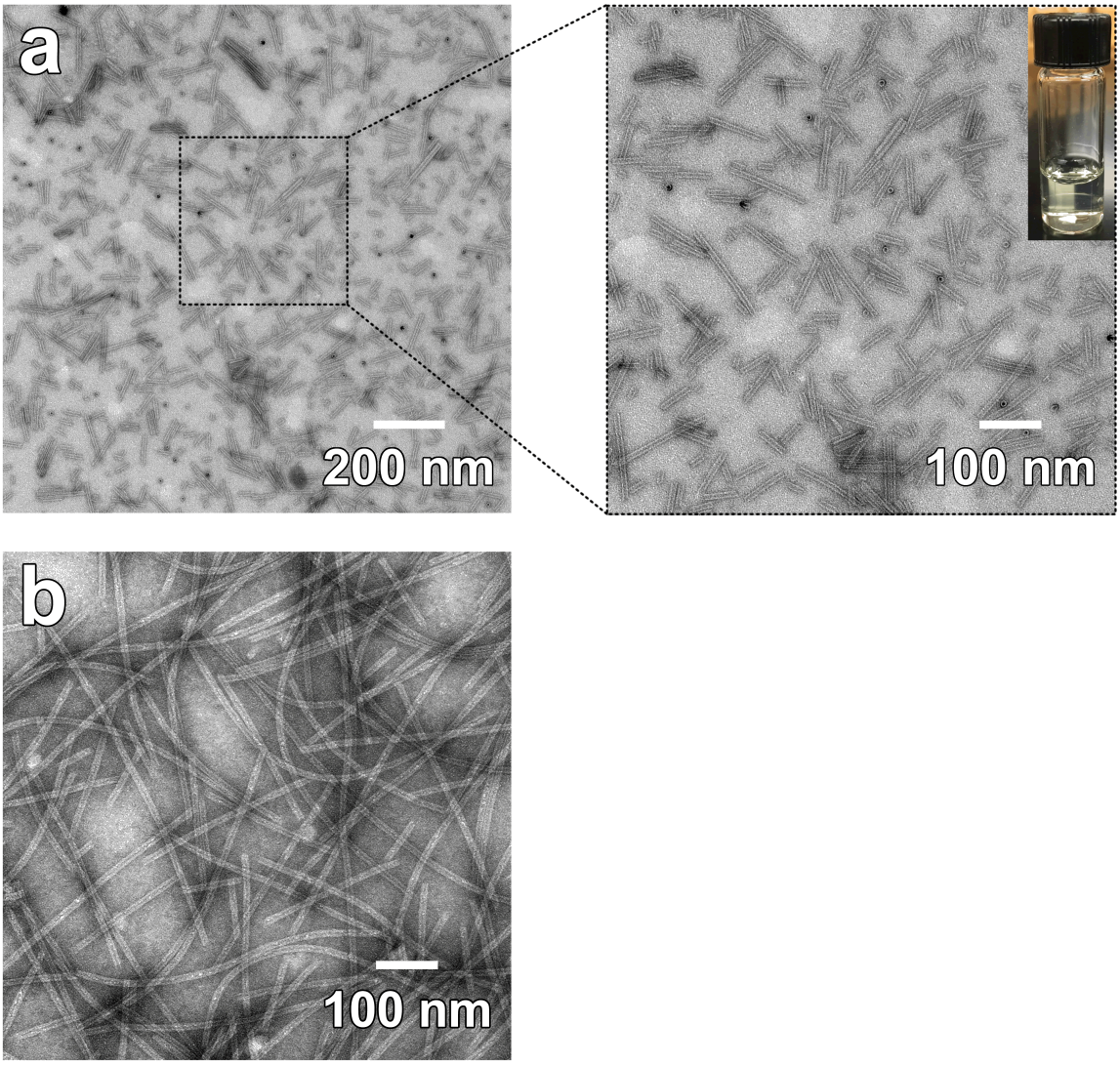

Figure S2. TEM images of (a) **NDI-Bola** (2 mM) in water diluted from 20 mM solution after continuous sonication at 20% amplitude for 3 min, and (b) **NDI-Bola** (2 mM) diluted from recovered 20 mM **NDI-Bola** at ambient temperature for 72 h. Inset: **NDI-Bola** (2 mM) in water after sonication. The tubular structure of **NDI-Bola** significantly recovered after 72 h of incubation compared with shortened **NDI-Bola** nanotubes with lengths of 50-100 nm after sonication, through the reversible self-assembly of **NDI-Bola**.

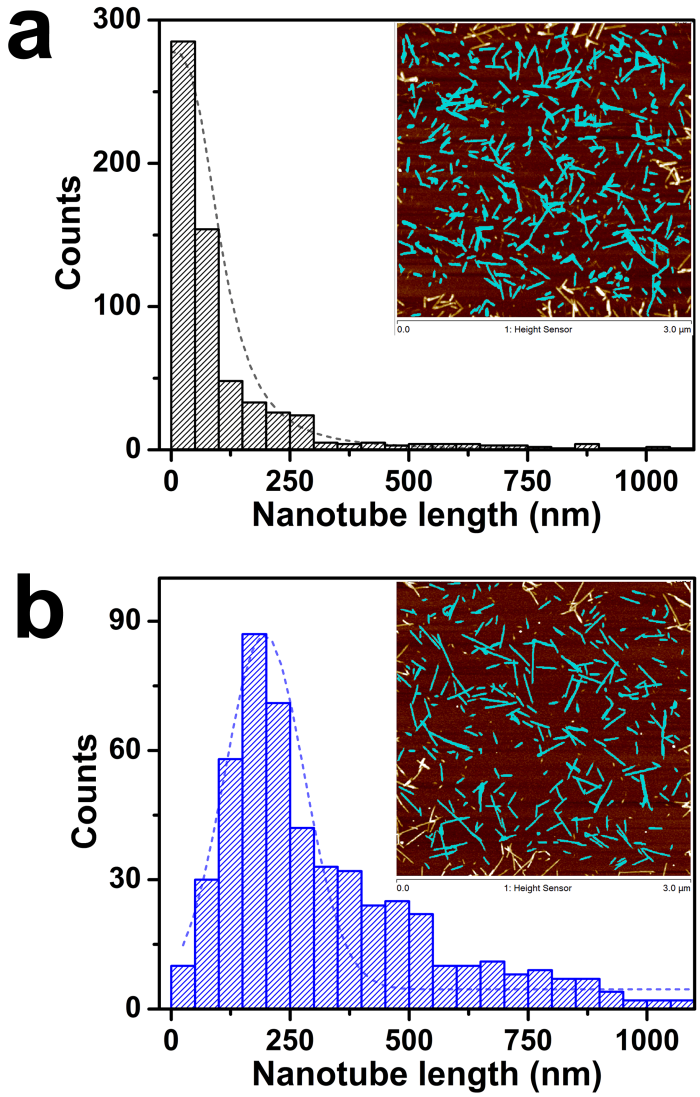

Figure S3. Histograms of length distributions of **NDI-Bola** nanotubes sonicated at (a) 20% and (b) 10% amplitudes, extracted from AFM images. (a) After sonication at 20% amplitude, the length distribution histogram of shortened **NDI-Bola** nanotubes partially fits to a logistic curve, with 72% of the nanotubes were shorter than 100 nm. (b) The length distribution of **NDI-Bola** nanotubes sonicated at 10% amplitude fits one Gaussian peak at 145 nm, and 48% of the nanotubes have a length between 100 and 200 nm. Insets show representative AFM images for the calculation of length distribution.

Length distributions were extracted from AFM images. After sonication, diluted **NDI-Bola** (1 mM) was drop-casted on freshly cleaved mica. AFM images (3.0 × 3.0 μm) were taken at a line frequency of 0.5 Hz and at a resolution of 512 × 512 pixels. Obtained AFM images were analyzed with Bruker NanoScope Analysis (version 1.40) software. Only nanotubes that appeared to be single filaments, rather than pairs or higher-order aggregates, were selected for particle analysis. Then, divided by the diameter of corresponding **NDI-Bola** nanotubes, area histogram extracted form particle analysis was converted into length distribution. For each sample, five images were used for statistical analysis with no less than 100 measurements in each case.

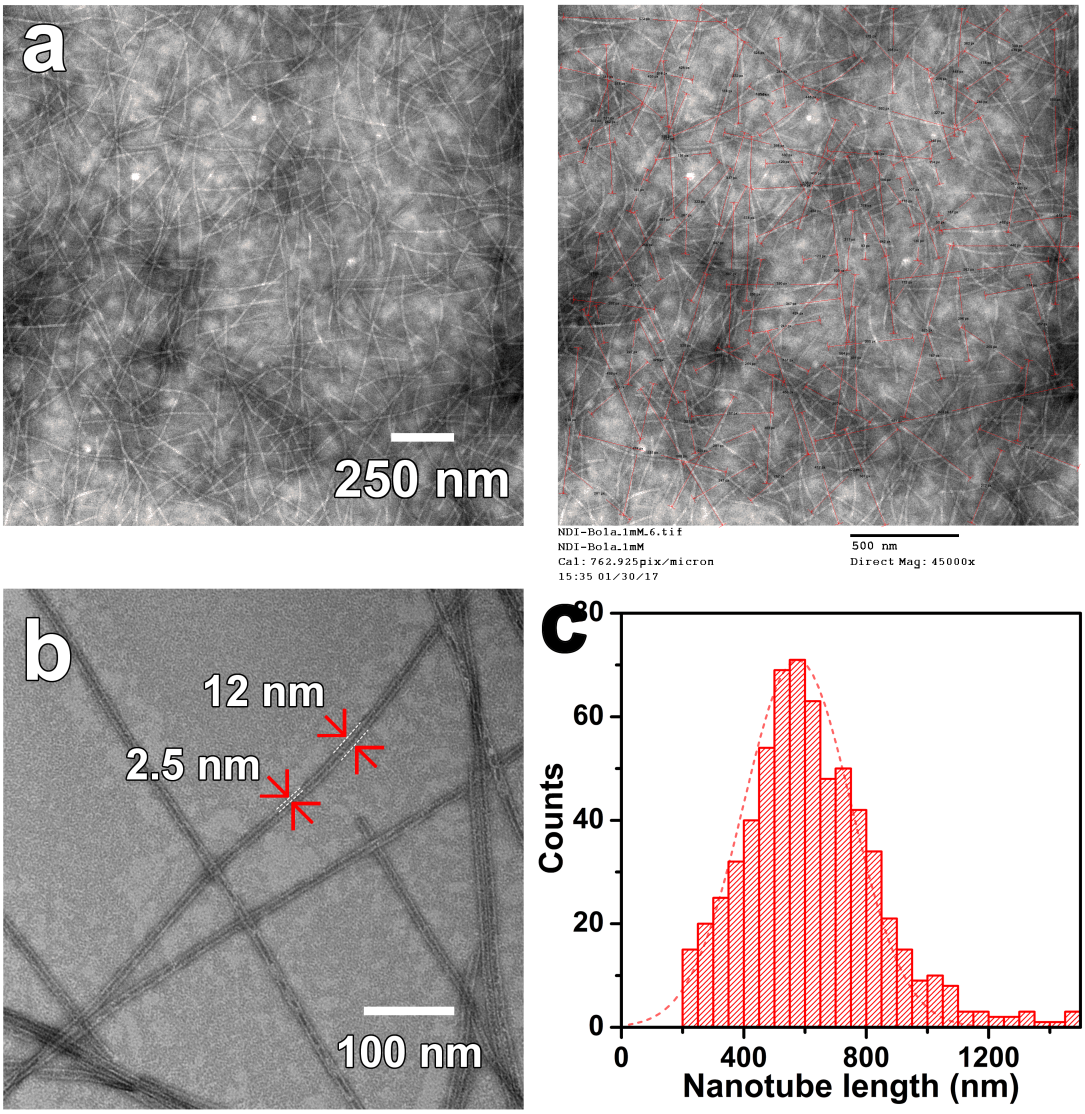

Figure S4. (a, b) TEM images of preformed **NDI-Bola** (2 mM) nanotubes in water (carbon-coated copper grid). 2% (w/w) uranyl acetate as the negative stain. (c) The length distribution histogram measured from (a) and the dashed curve fits to Gaussian function. The length of preformed **NDI-Bola** nanotubes fits one Gaussian peak at 575 nm, and 54% of the nanotubes have a length between 500-700 nm.

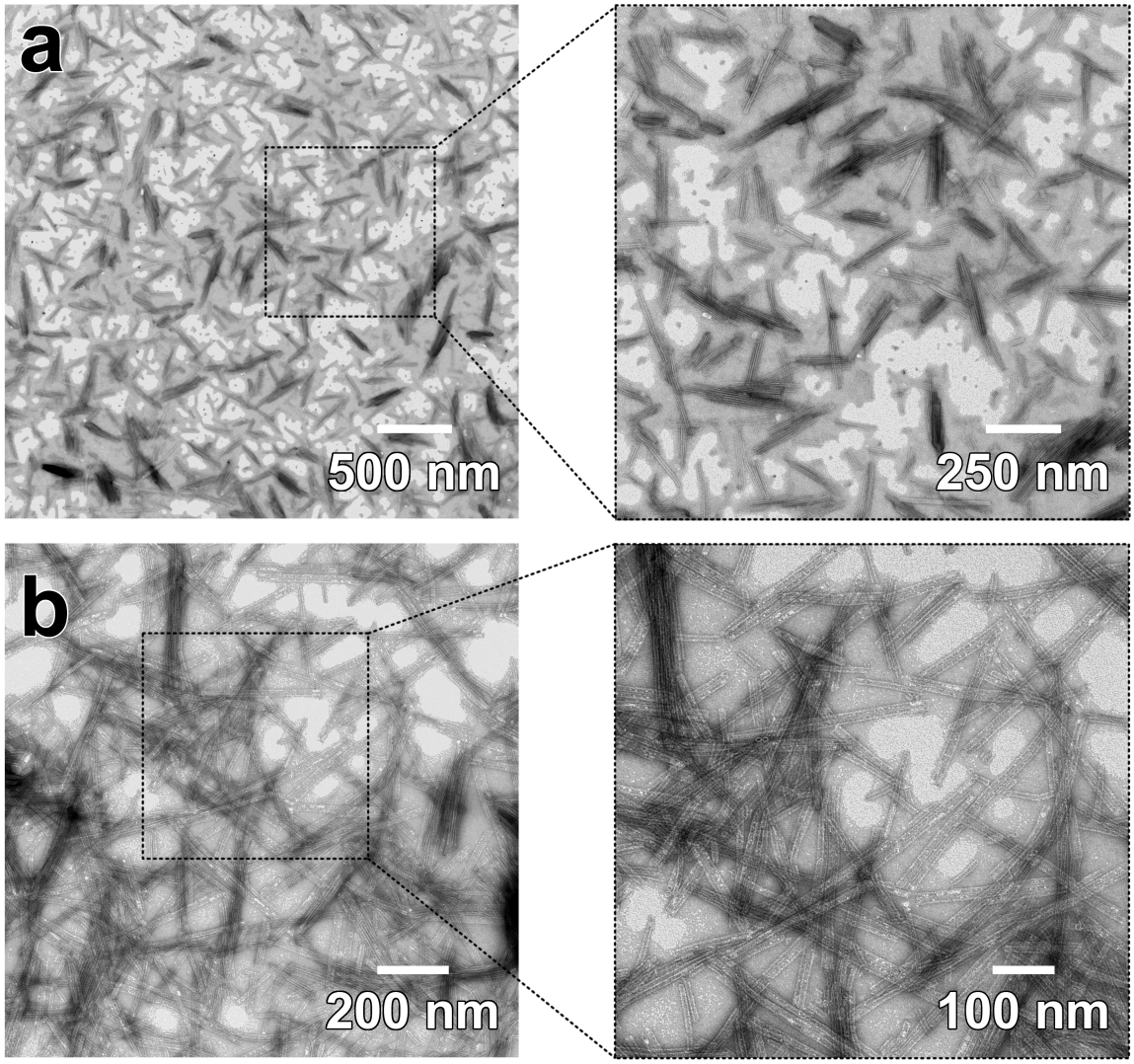

Figure S5. TEM images of **NDI-Bola** (2 mM) in water (a) after sonication at 30% amplitude for 3 min and (b) the resulting **NDI-Bola** fragments aged for 3 d. Sonication at 30% amplitude not only broke the original tubular structure, but also propelled the formation of higher-order bundles of shortened **NDI-Bola** nanotubes.

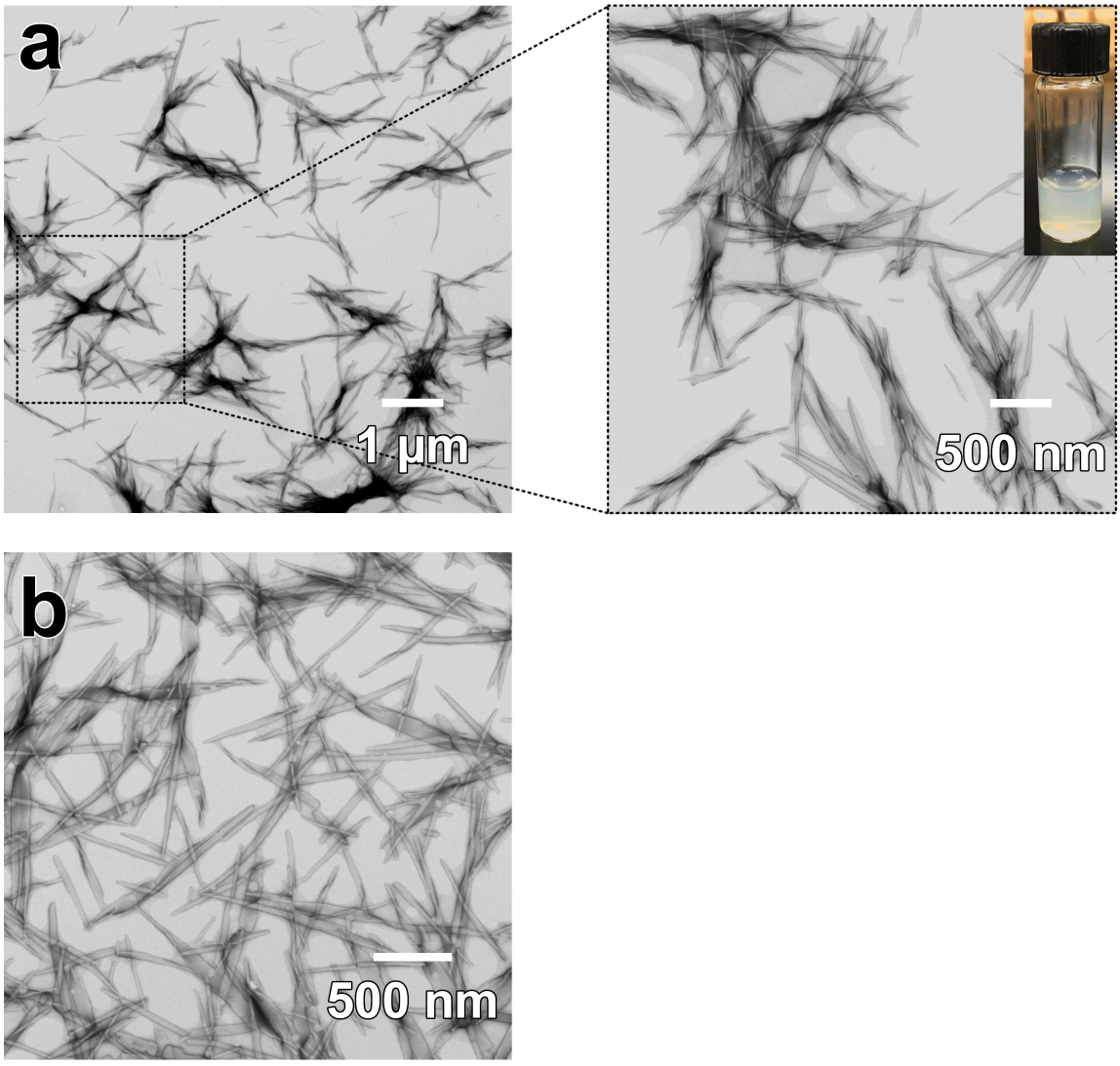

Figure S6. TEM images of **NDI-Bola** (2 mM) in water (a) after sonication at 60% amplitude for 3 min and (b) the resulting **NDI-Bola** fragments aged for 5 d. Inset: **NDI-Bola** (2 mM) in water after sonication. Inset: The transparent solution of elongated **NDI-Bola** nanotubes became cloudy after sonication. TEM observation shows sonicating at 60% amplitude caused irreversible dissociation of NDI-Bola nanotubes and the formation of nanosheet bundles.

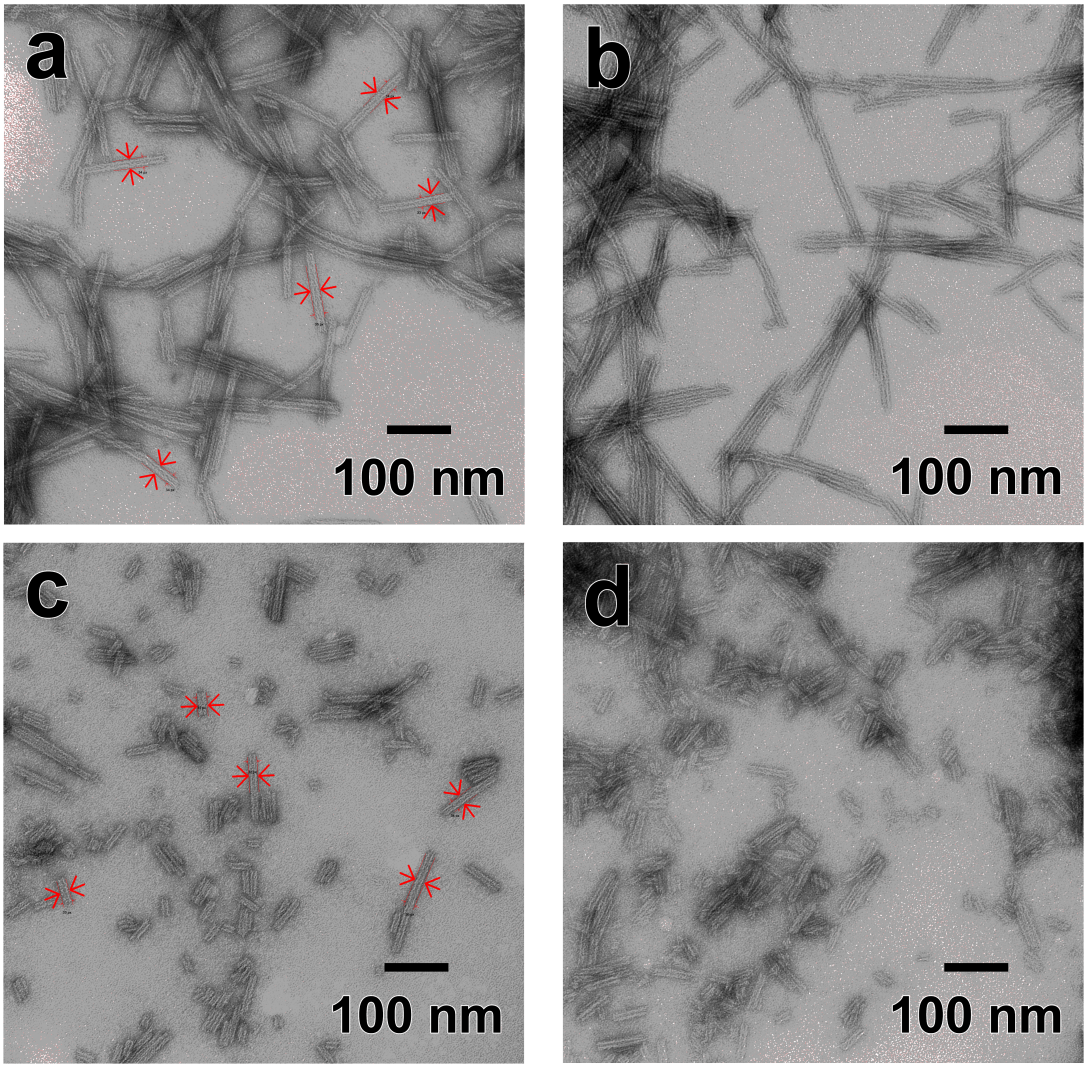

Figure S7. TEM images of the co-assembly of shortened **NDI-Bola** (2 mM) nanotubes and PCT-SO_3Na (0.4 mM) in water. **NDI-Bola** (20 mM) was sonicated at (a, b) 10% and (c, d) 20% amplitudes for 3 min, then, PCT-SO_3Na was added to the sonicated nanotube fragments, resulting in immediate formation of transparent hydrogels. After incubating for (a, c) 3 and (b, d) 7 days, the resulting polymer-nanotube composites were diluted with deionized water and centrifuged (5000 rpm). The supernatants were decanted off, and pure polymer-nanotube composites as pellets were redispersed in water

(concentration of **NDI-Bola** was 2 mM) for TEM tests. Arrows indicate examples for diameter measurements. Diameters of **NDI-Bola**/PCT-SO_3Na nanotubes are (a) 17.4 ± 0.4 and (c) 17.4 ± 0.8 nm, respectively.

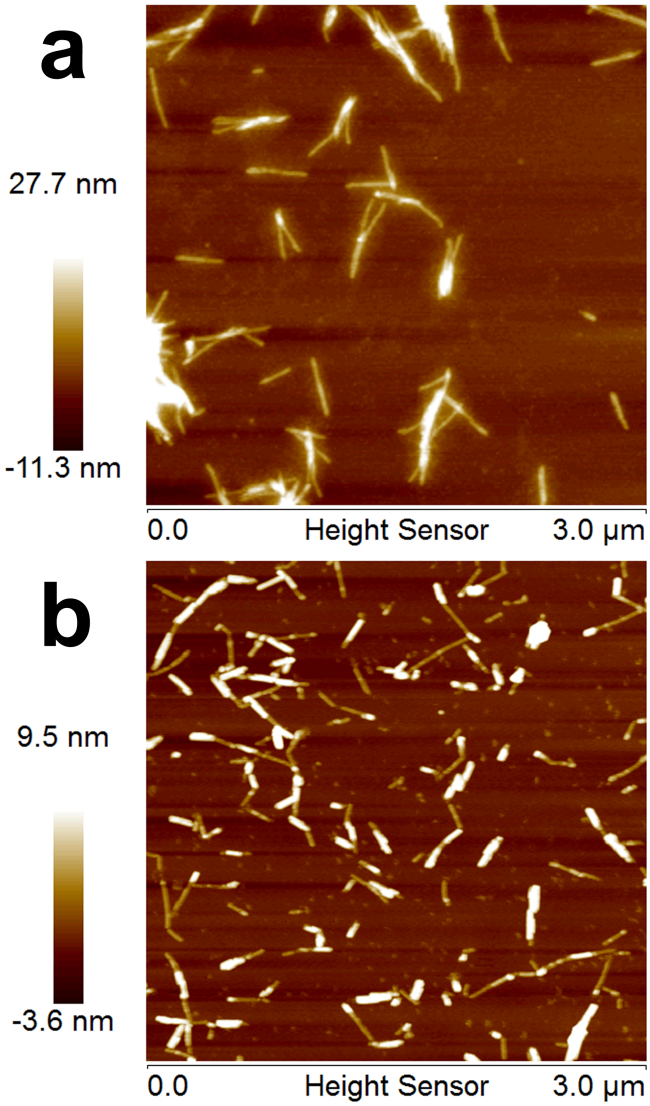

Figure S8. AFM images of the co-assembly of **NDI-Bola** (2 mM) nanotubes, fragmented by sonication, and PCT-SO_3Na (0.4 mM) in water. **NDI-Bola** (20 mM) was sonicated at (a) 10% and (b) 20% amplitudes for 3 min, prior to mixing with PCT-SO_3Na to afford **NDI-Bola**/PCT-SO_3Na composites. AFM images were taken from composites after aging for 3 d. Lengths of **NDI-Bola**/PCT-SO_3Na composites, calculated from AFM images, are (a) 167.7 ± 44.3 and (b) 67.3 ± 23.2 nm, respectively.

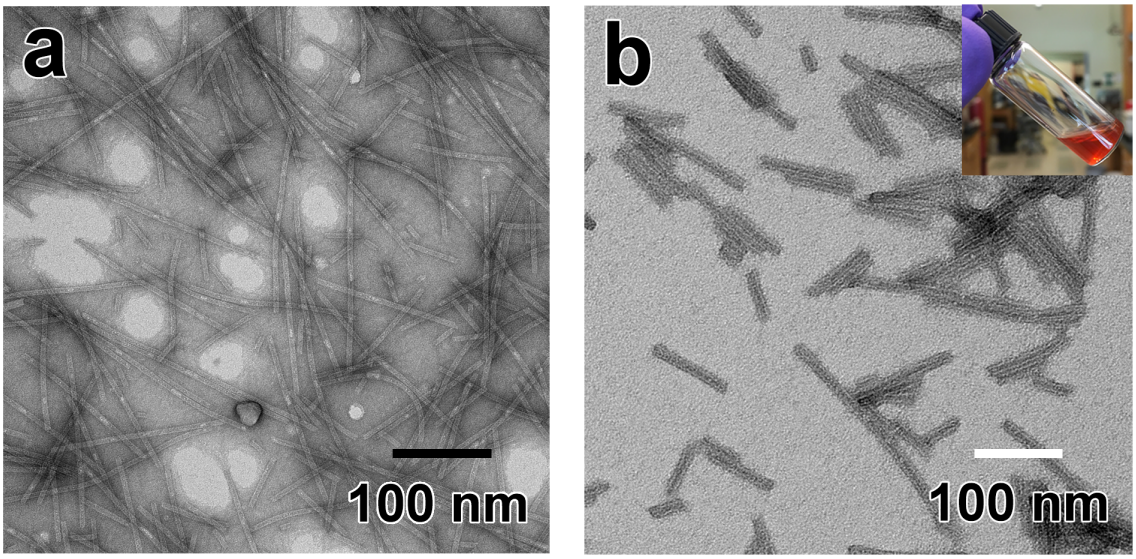

Figure S9. NDI-Bola (20 mM) was sonicated at 10% amplitude for 3 min. Then, **NDI-Bola** (5 mM) diluted from the sonicated **NDI-Bola** (20 mM) fragments was treated with PCT-SO_3Na (10:2 **NDI-Bola**/PCT-SO_3Na). TEM images of (a) **NDI-Bola** (5 mM) and **NDI-Bola**/PCT-SO_3Na aged for 5 d before imaging. Inset: Adding PCT-SO_3Na to the diluted **NDI-Bola** fragments (5 mM) didn't product hydrogels.

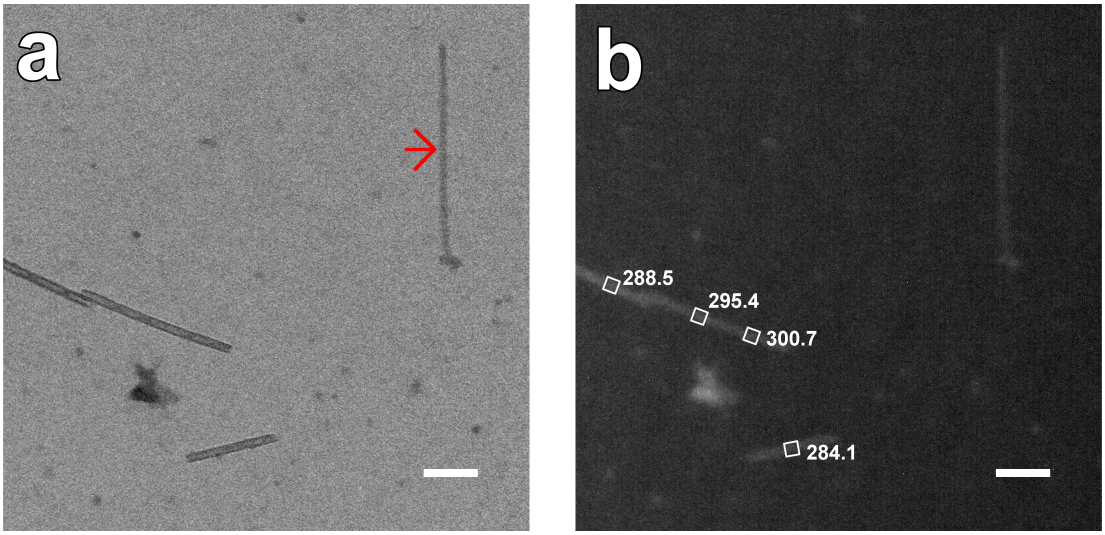

Figure S10. Mass-per-length (MPL) measurements of **NDI-Bola**/PCT-SO_3Na (10% amplitude) nanotubes. Arrows in bright-field TEM images (a) indicate TMV rods. Examples of MPL values (kDa/nm) determined for segments enclosed in white squares are shown in dark-field TB-TEM images (b). Scale bars, 100 nm.

In bright-field TEM images, unstained nanotubes appeared as tubular structures with a bright hollow center, which is entirely different from the appearance of TMV as straight rods with a solid dark center. Only nanotubes and TMV that appeared to be single filaments were selected for MPL calculation. The MPL value of **NDI-Bola** nanotubes is 187.5 ± 12.2 kDa/nm, in contrast, that of **NDI-Bola**/PCT-SO_3Na (10% amplitude) nanotubes is 286.5 ± 17.0 kDa/nm, indicating the formation of polymer wrapping layer around **NDI-Bola** nanotubes. MPL values were calculated form TB-TEM images.[5,6] Firstly, image intensities were integrated over rectangular areas (25 × 25 nm) centered on nanotubes (I_N) and over same areas of background on either side of nanotubes (I_B). The intensity value of nanotubes ($I_{Nanotube}$) was calculated as the average of the quantities of

(I_N-I_B). Then, the intensity value of TMV (I_{TMV}) was calculated by following the same procedure. Finally, MPL values were calculated as $131 \times I_{Nanotube}/I_{TMV}$.

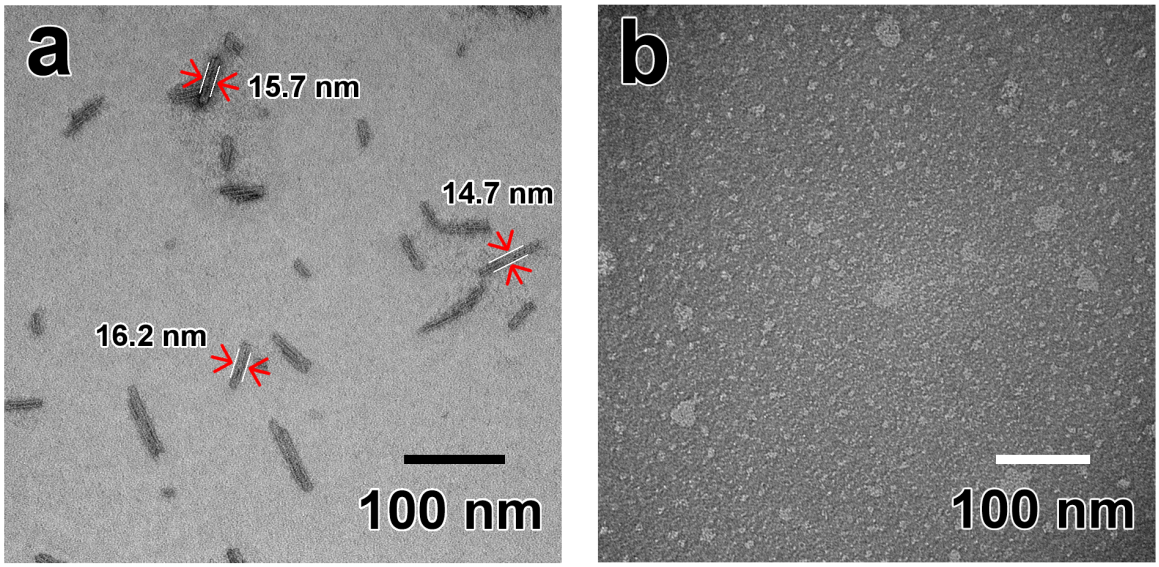

Figure S11. TEM images of (a) co-assembly of shorted **NDI-Bola** (2 mM) nanotubes and Cur-SO_3Na (0.2 mM) aged for 3 d and (b) Cur-SO_3Na (6 mM) in water. **NDI-Bola** was sonicated at 20% amplitude for 3 min, prior to mixing with Cur-SO_3Na to afford **NDI-Bola**/Cur-SO_3Na composites. The diameter of NDI-Bola/Cur-SO_3Na nanotubes is 15.4 ± 1.2 nm.

Curdlan, as the simplest linear polysaccharide of (1−3) linked β-D-glucose units, has been used as supramolecular wrapping agent with inherent bio-compatibility.[7,8] Natural curdlan adopts a helical conformation in anhydrous form. The selective modification of 6-OH group, which always exists on the exterior surface of the curdlan helix, effectively improves its undesirable water solubility due to the lack of side glucoses.[9] In our research, cardlan was sulfated with piperidine-*N*-sulfonic acid in DMSO to give Cur-SO_3Na. After sulfation, imported electrostatic repulsion among the anionic charges stretches the rigid helix of Cur-SO_3Na to a loosely tied triple strand or to a single strand in water.[10,11] After sonicating at 20% amplitude for 3 min, shortened **NDI-Bola** nanotubes were treated with Cur-SO_3Na resulting in immediate formation of hydrogels

similar to **NDI-Bola**/PCT-SO_3Na composites. The length distribution of **NDI-Bola**/Cur-SO3Na nanotubes remained stable after 3 d, indicating **NDI-Bola** fragments after sonication were coated by Cur-SO_3Na, which prohibited the recovery of the full length of **NDI-Bola** nanotubes.

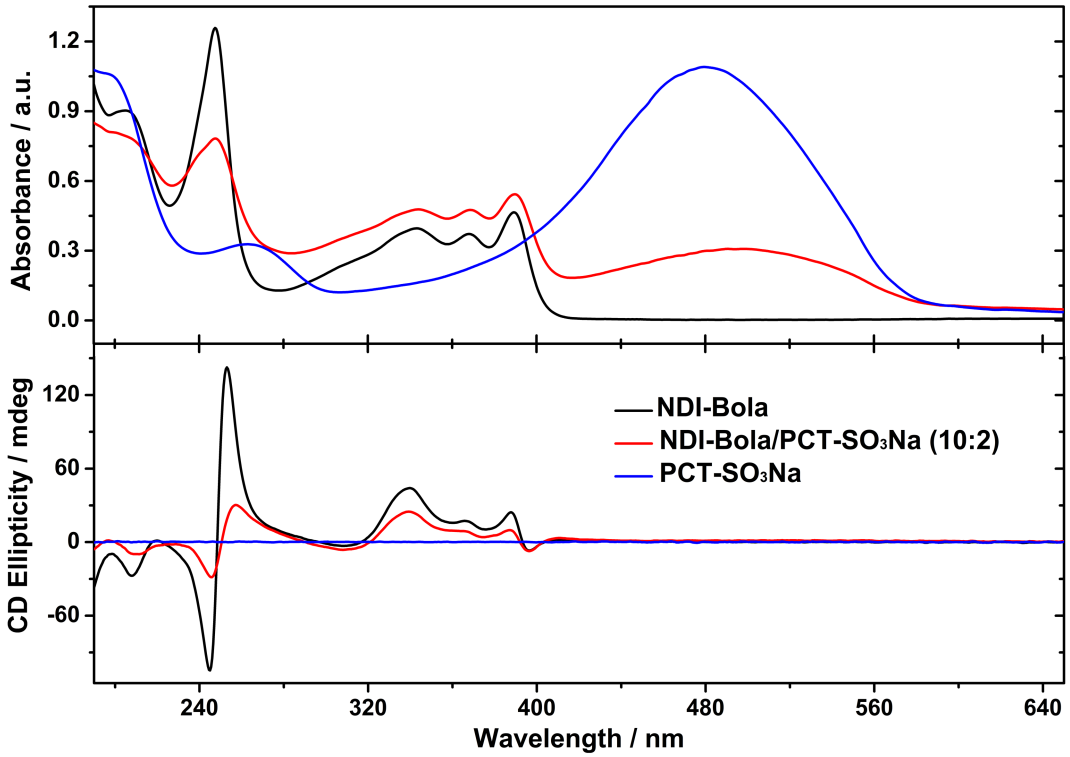

Figure S12. UV-Vis and CD spectra of **NDI-Bola** (500 μM, black), **NDI-Bola**/PCT-SO_3Na (10:2, 1 mM in **NDI-Bola**, red), and PCT-SO_3Na (500 μM, blue) in water. The UV-Vis spectrum of **NDI-Bola**/PCT-SO_3Na (10:2) showed characteristic peaks of **NDI-Bola** nanotubes at 342, 368, 389 (band I), and 248 nm (band II), and the broad absorption in 420-600 nm resulting from PCT-SO_3Na, which demonstrates the successful coassembly of **NDI-Bola** nanotubes and PCT-SO_3Na. The CD spectrum of PCT-SO_3Na displayed a flat profile, while that of **NDI-Bola**/PCT-SO3Na composites inherited the positive couplet centered at 248 nm of **NDI-Bola** nanotubes resulting from the right-handed, P-type helical arrangement of *y*-polarized transition dipoles of **NDI-Bola**,[3] which further demonstrates the long-range orientation of **NDI-Bola** molecules has been preserved after mixing with PCT-SO_3Na.

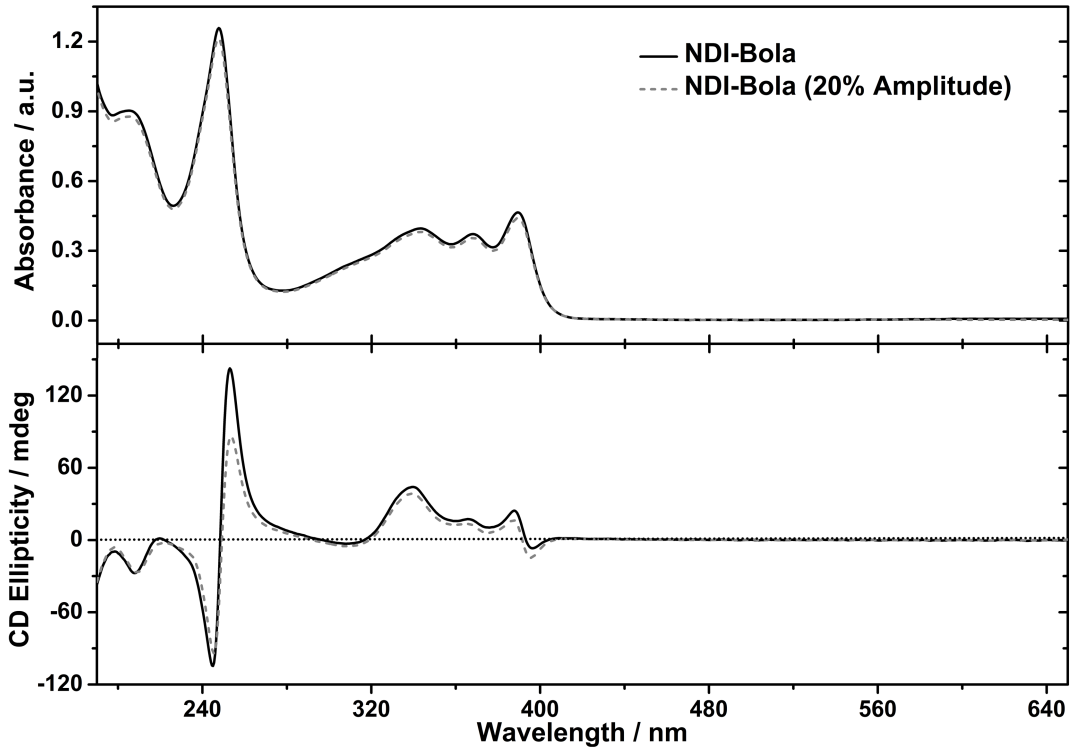

Figure S13. UV-Vis and CD spectra of **NDI-Bola** nanotubes (black) and fragmented **NDI-Bola** nanotubes by sonication at 20% amplitude (dashed grey). The concentration of **NDI-Bola** in all samples was 500 μM.

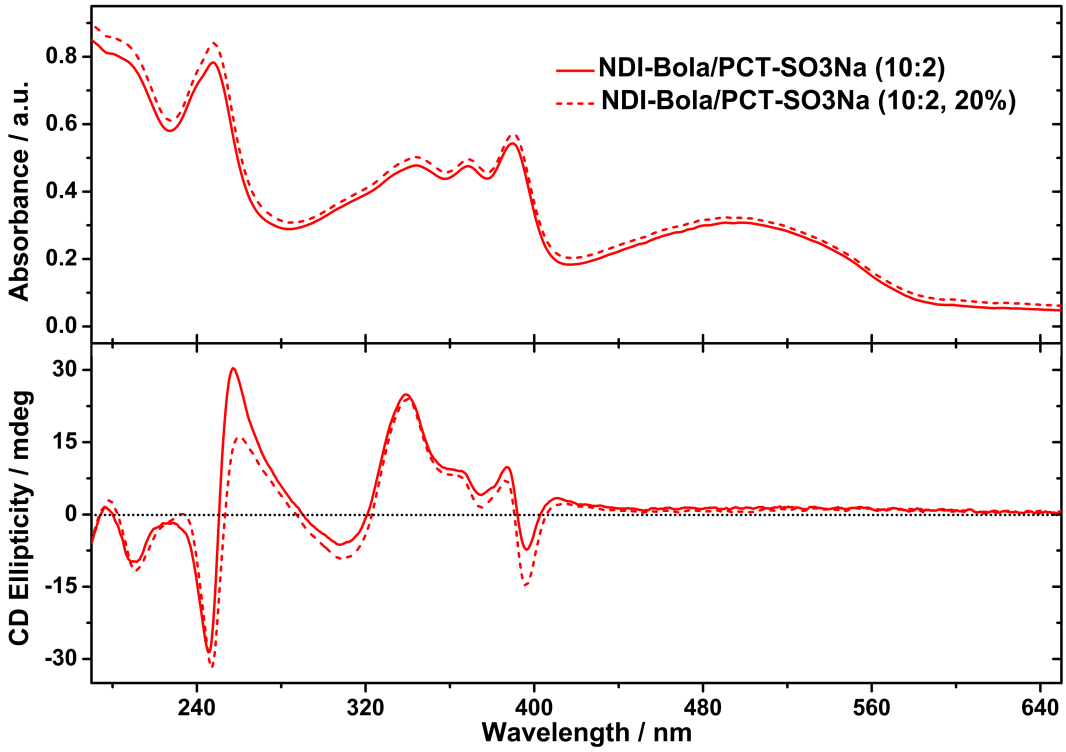

Figure S14. UV-Vis and CD spectra of **NDI-Bola**/PCT-SO_3Na (10:2) composites (red) and **NDI-Bola**/PCT-SO_3Na (10:2, 20%) composites (dashed red) prepared from fragmented **NDI-Bola** nanotubes by sonication at 20% amplitude. The concentration of **NDI-Bola** in all samples was 1 mM.

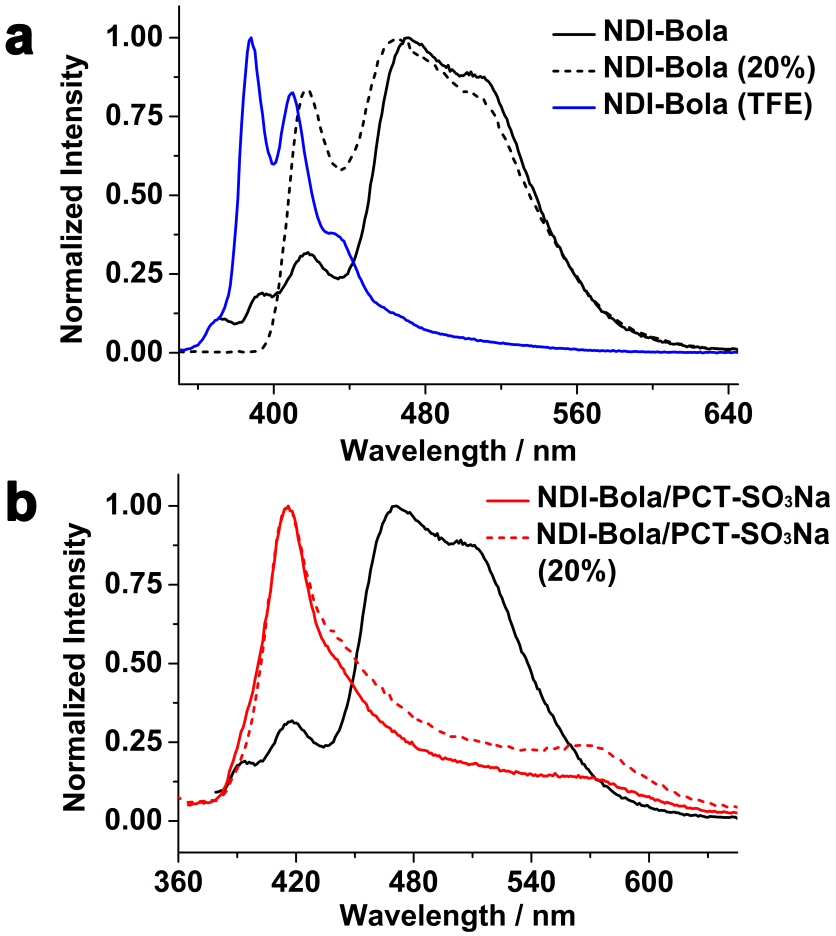

Figure S15. Steady state fluorescence spectra of (a) **NDI-Bola** nanotubes and (b) **NDI-Bola**/PCT-SO_3Na composites. (a) **NDI-Bola** in water (black), fragmented **NDI-Bola** by sonication at 20% amplitude (dashed black), and monomolecular **NDI-Bola** in TFE (blue). (b) **NDI-Bola** in water (black), **NDI-Bola**/PCT-SO_3Na (10:2) composites in water (red) and with 20% amplitude sonication (dashed red). For **NDI-Bola**/PCT-SO_3Na (20%) composites, self-assembled **NDI-Bola** nanotubes were fragmented by sonication at 20% amplitude, prior to mixing with PCT-SO_3Na. Except for **NDI-Bola** in TFE (100 μM), the concentration of **NDI-Bola** in all samples was 2 mM. All samples were excited at 330 nm.

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