Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Co-Assembly of a Multicomponent Network of Nanofiber-Wrapped Nanotubes.

*McKensie L. Mason,[1†] Tao Lin,[1†] Jenae J. Linville, [†] and Jon R. Parquette[†]**

[†]Department of Chemistry, The Ohio State University, 100 W. 18th Ave. Columbus, Ohio 43210

[1]Both authors contributed equally to this work.

Email: parquette.1@osu.edu

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Experimental Section.

General Methods. Atomic force microscopy (AFM) was conducted on a Bruker AXS Dimension Icon Atomic Force Microscope. Transmission Electron Microscopy (TEM) was carried out with Technai G2 Spirit instrument operating at 80 keV. ^{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. Circular dichroism with corresponding UV-Vis absorption measurements were performed in a JASCO J-815 CD Spectrometer under nitrogen atmosphere in a 1mm path length quartz cuvette at 25°C. All pH measurements were obtained using a Mettler Toledo MP 125 pH meter with InLab Micro pH probe. Fluorescence excitation and emission experiments were performed on a Shimadzu RF-5301PC Spectrofluorophotometer in a triangular or 3mm path length quartz cuvette. UV-vis experiments were performed on a Shimadzu UV-2450 Spectrophotometer in a 1mm cuvette. Zeta potential measurements were obtained using a Malvern Zetasizer NanoZS systems with folded capillary zeta cells (DTS1070).

Atomic Force Microscopy (AFM): AFM images were collected on Bruker AXS Dimension Icon Atomic Force Microscope in ScanAsyst mode using the SAA-HPI-SS probe from Bruker with a 1 nm tip end radius and a 0.25 N/m spring constant. Samples (1 mM) were deposited onto freshly cleaved mica for 1 min, rinsed with water for 10 s, then dried with nitrogen gas. Images were analyzed with Bruker's software.

Transmission Electron Microscopy (TEM): Samples (1 mM) were dropped on carbon-coated copper grids (Ted Pella, Inc.) for 1 min. After removal of excess solution, the sample grid was negatively stained

with 2% (w/w) uranyl acetate solution for 30 s. The dried specimen was observed with Technai G2 Spirit TEM instrument operating at 80 keV. Images were analyzed with FIJI imaging software.

Zeta Potential Measurements: Zeta potential measurements were taken of samples diluted from 1 mM to 0.5 mM in water folded capillary zeta cells (DTS1070) on a Malvern Zetasizer NanoZS system, after recording the pH for each sample. Measurements were taken in triplicate and the mean and standard deviation were reported. Data was analyzed using Malvern Zetasizer Software.

Base Titration: Base titration of **2** (0.5 mM) with 0.01 mM NaOH after first adding HCl to decrease the starting point to a pH of 2.83. Known volumes of NaOH were added while recording the pH to obtain a titration curve. The equivalence point was determined by examining the first derivative of the titration curve. The point at which the slope was most positive corresponded to the equivalence point, and the pKa value 3.22 was assigned to the half-equivalence point associated with the equivalence point at pH 5.82. According to the Henderson-Hasselbalch equation, pH = pKa when the concentration of acid and base are equal (at the half-equivalence point), so the pKa of the carboxylic acid proton of **2** was calculated to be about 3.22.

Powder X-ray Diffraction (PXRD): The PXRD spectrum was taken on a Bruker D8 Advance PXRD Instrument is equipped with LYNXEYE XE-T detector and data collection was taken via Kα1 radiation (λ = 1.5418 Å). The sample was spread onto a powder XRD sample holder and data was collected with a sampling interval of 0.01° per step and a counting rate of 2s per step.

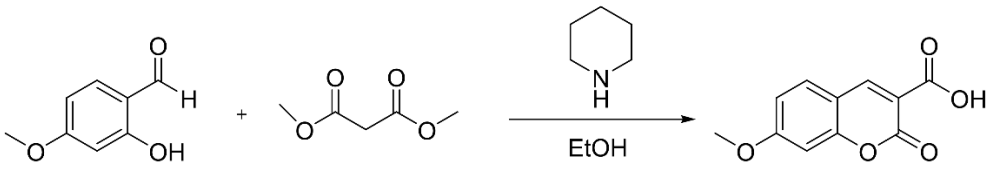
FRET Efficiency Calculations: Energy transfer efficiencies were calculated using 1:

$$E = 1 - \frac{F_{DA}}{F_D} \quad \text{(eq. 1)}$$

Where E is the energy transfer efficiency, F_D is the fluorescence intensity of the donor, and F_{DA} is the fluorescence intensity of the donor in the presence of the acceptor.[1-3] FRET efficiencies reported were generated from fluorescence intensities at 415 nm, averaged from 3 separate measurements per sample.

Synthesis of EFEK(DAC)-NH_2 (1) and 7-(*N*,*N*-diethylamino)-3-coumarin carboxylic acid (DAC-CO_2H). Prepared according to previously published methods.[4]

Synthesis of 7-Methoxycoumarin-3-CO_2H (MC-CO_2H): The synthesis of MC-CO_2H was adapted from the procedure previously used to synthesize DAC-CO_2H[4] following the reaction scheme shown in Scheme S1. A solution of 2-hydroxy-4-methoxybenzaldehyde (3.82 g, 25.11 mmol, 1 eq.) and dimethyl malonate (6.07 mL, 52.83 mmol, 2.1 eq.) in ethanol (60 mL) was prepared. Piperidine (2 mL) with a catalytic amount of glacial acetic acid (1 drop) was added to the reaction mixture. After 12 h at reflux at 85 °C, 1 N NaOH (aq.) solution was added with vigorous stirring, producing a yellow precipitate. The mixture was heated to reflux for 20 min, until dissolved. The solution was cooled to rt and 2 N HCl (aq.) was added to the mixture until a pH of ~2. The resulting pale yellow solid was collected by filtration and recrystallized with ethanol to obtain the pale yellow crystalline product (2.70 g, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.22 (1H, s), 8.88 (1H, d, J = 0.4 Hz), 7.66 (1H, d, J = 8.8 Hz), 7.04 (1H, dd, J = 8.8, 2.4 Hz), 6.96 (1H, d, J = 2.3 Hz), 3.98 (3H, s); ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.28, 164.54, 163.06, 157.09, 151.22, 131.71, 115.15, 112.34, 110.67, 100.81, 56.30. ESI-MS calculated for $C_{11}H_8O_5$ (M+H) 221.0372, found 221.0366.

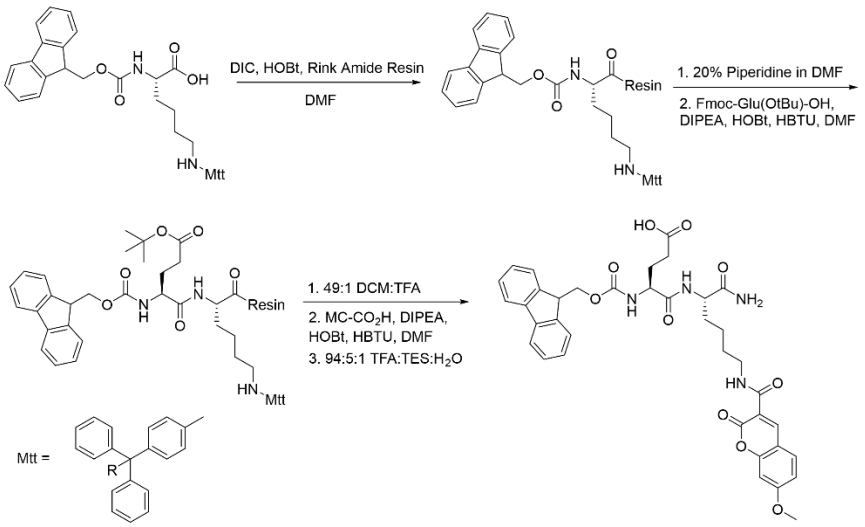


Scheme S1. Synthesis of MC-CO_2H.

Synthesis of Fmoc-EK(MC) (2):

The dipeptide was manually prepared using Fmoc solid-phase peptide synthesis on rink amide resin (loading 0.4 mmol/g), as shown in Scheme S2. Amide-coupling steps were accomplished with standard techniques for all amino acids: Fmoc-amino acid, 1,3-diisopropylcarbodiimide (DIC), and 1-hydroxybenzotriazole (HOBt) or HOBt, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium

hexafluorophosphate (HBTU), and diisopropylamine (DIPEA) (3 equivalents each relative to resin) in DMF for 2 h. A solution of 20 % piperidine in DMF was used for Fmoc removal and 1 % TFA in dichloromethane was used for Mtt group deprotection. The final MC-dipeptide conjugates were cleaved from the resin by the treatment with TFA/triethylsilane/water (94 / 5 / 1) at room temperature for 2 h. The crude peptides were precipitated with diethyl ether and purified by reversed-phased HPLC on preparative Waters XBridge C8 column eluting with a gradient of CH_3CN/water containing 0.1 % TFA (30%-80% CH_3CN over 40 minutes) and stored as lyophilized powders at -18°C. Yield on the following scale was 83 mg, 0.12 mmol, 30%): Rink amide resin: (930 mg, 0.4 mmol, 1 eq.), Fmoc-Lys(MTT) (750 mg, 1.2 mmol, 3 eq.), Fmoc-Glu(OtBu): (510 mg, 1.2 mmol, 3 eq.). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.73 (1H, s), 8.55 (1H, t, *J* = 5.7 Hz), 7.74-7.85 (3H, m), 7.64 (2H, t, *J* = 7.6 Hz), 7.48 (1H, d), 7.23-7.35 (4H, m), 6.94-7.02 (3H, m), 4.09-4.19 (4H, m), 3.93-3.99 (1H, m), 3.82 (1H, s), 3.18-3.23 (2H, m), 2.19 (2H, t, *J* = 7.8 Hz), 1.79-1.88 (1H, m), 1.56-1.73 (2H, m), 1.42-1.50 (3H, m), 1.21-1.25 (2H, m),; ^{13}C NMR (400MHz, DMOS-d6) δ 174.43, 173.91, 171.66, 164.81, 161.76, 161.34, 156.58, 148.14, 144.36, 144.18, 144.15, 131.95, 128.08, 127.54, 125.75, 120.54, 115.37, 114.07, 112.59, 100.72, 66.16, 56.69, 54.46, 52.59, 47.11, 32.22, 30.71, 29.19, 27.75, 23.14. ESI-MS calculated for $C_{37}H_{38}N_4O_{10}$ (M+H) 699.2588, found 699.2565.



Scheme S2. Synthesis of Fmoc-EK(MC), **2**, via solid-phase peptide synthesis.

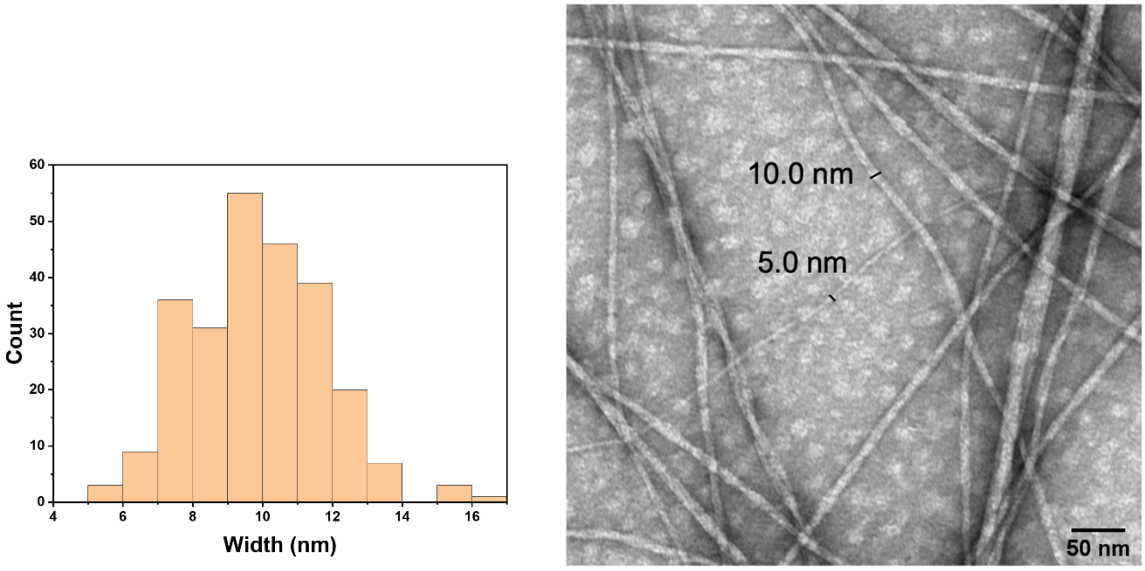


Figure S1. (left) Histogram generated from 250 TEM measurements of the widths of nanofibers of Fmoc-EK(MC) (**2**) using FIJI imaging software. (right) TEM image of Fmoc-EK(MC) **2** nanofibers, aged for 2 weeks at 1 mM, pH 7.0. Width measurements were acquired using FIJI imaging software.

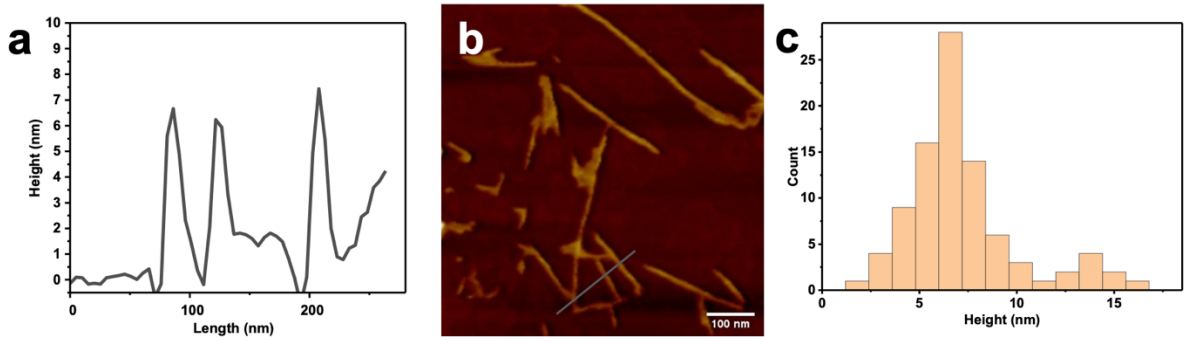


Figure S2. Fmoc-EK(MC) (**2**) nanofibers aged for 3 days at 1 mM, pH 4 (a) cross-sectional height profiles measured from the AFM image in (b). (b) representative AFM image of nanofibers of **2**. (c) Histogram of measured heights from AFM images from ~90 measurements demonstrating a bimodal distribution correlating to single fibrils (avg. height 6 nm) and two fibrils wrapped into a fiber (avg. height 13 nm). The single fibrils are more prevalent in this sample due to the shorter (3-day) aging time.

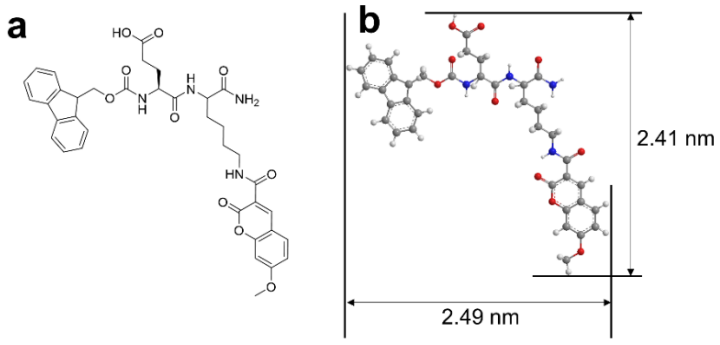


Figure S3. (a) ChemDraw and (b) Chem3D structures of Fmoc-EK(MC) **2**. Length measurements were taken in Chem3D of the molecule in the shown flattened, extended conformation.

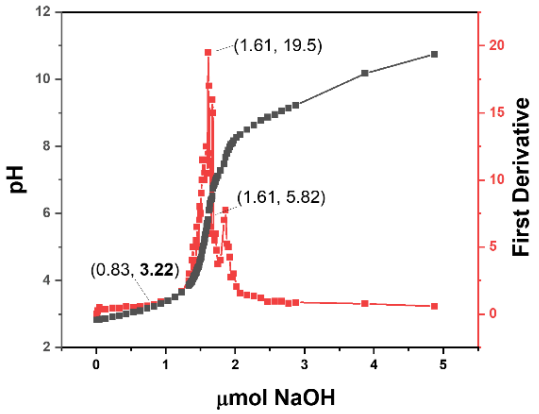


Figure S4. Titration of 0.5 mM **2** with 0.01 M NaOH in water. Initially added 0.5 M HCl to **2** to achieve a starting pH of 2.83, then added 1 μL volumes of 0.01 M NaOH solution while recording the pH. Analysis of the first derivative of the resultant titration curve provided the equivalence point at 1.61 μmol NaOH, and the pH at the half-equivalence point (the point where the concentration of acid and base are equal) was determined to be about 3.22. According to the Henderson-Hasselbalch equation, pH = pKa when the concentration of acid and base are equal, so the pKa of the carboxylic acid proton of **2** was calculated to be about 3.22.

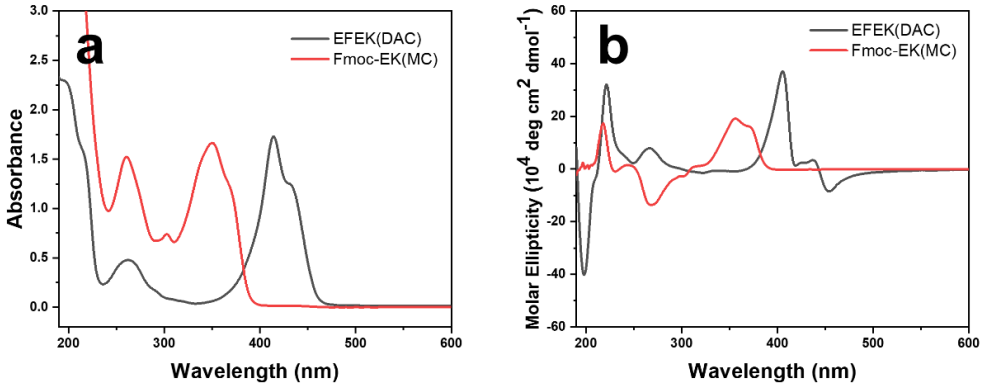


Figure S5. (a) UV-vis absorbance and (b) circular dichroism spectra of 0.5 mM **1** (black), aged at 1 mM for 24 h at pH 3.8, and 1 mM **2** (red) diluted from a 5 mM sample aged for 2 weeks. Spectra were taken in a 1 mm quartz cuvette.

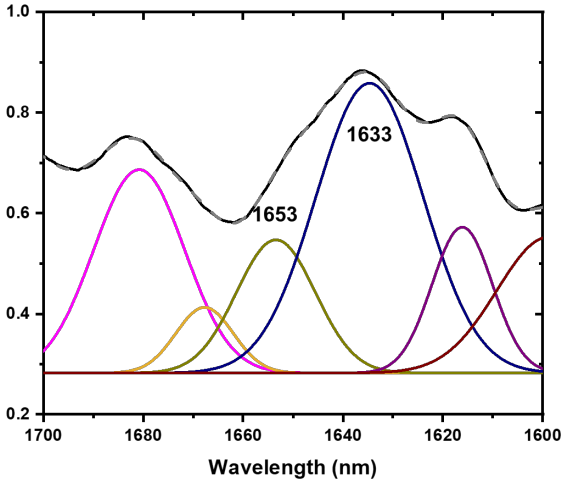


Figure S6. Deconvoluted FT-IR spectrum of **2**. The sample was prepared by assembling in D_2O (5 mM) over 1 week, then was lyophilized and redissolved in D_2O for analysis. Deconvolution of the FT-IR absorptions indicated the sample contained 75% β-sheet secondary structure.

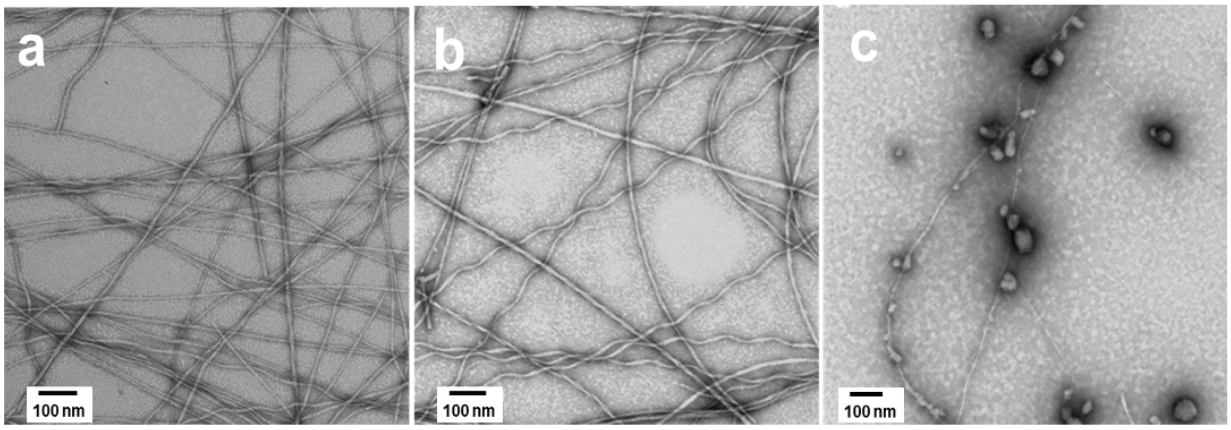


Figure S7. TEM images of Fmoc-EK(MC) (**2**) assembled in H_2O (pH 7.0) at various concentrations aged for 5-7 days at (a) 5 mM (b) 1 mM (c) and 0.5 mM. Samples were prepared for TEM analysis by dropping the solution onto carbon-coated copper grids for 1 min. After removal of excess solution, the sample grid was negatively stained with 2% (w/w) uranyl acetate solution for 30 s.

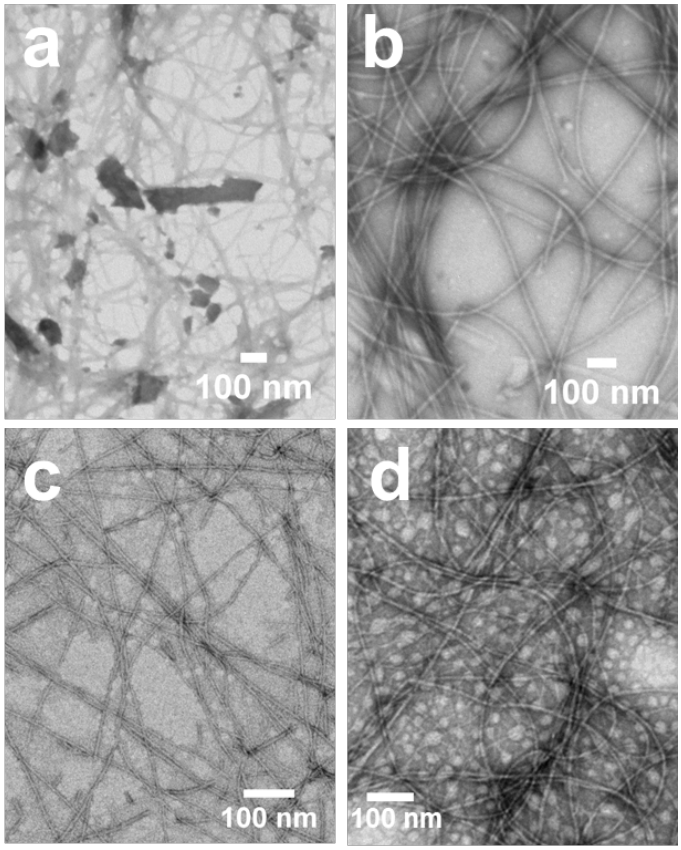


Figure S8. TEM images of **2** assembled in H_2O (1 mM) at various pH values for 5-7 days. (a) pH 2 (b) pH 4 (c) pH 6 (d) pH 8. Samples were prepared for TEM analysis by dropping the mixture onto carbon-coated copper grids for 1 min. After removal of excess solution, the sample grid was negatively stained with 2% (w/w) uranyl acetate solution for 30 s.

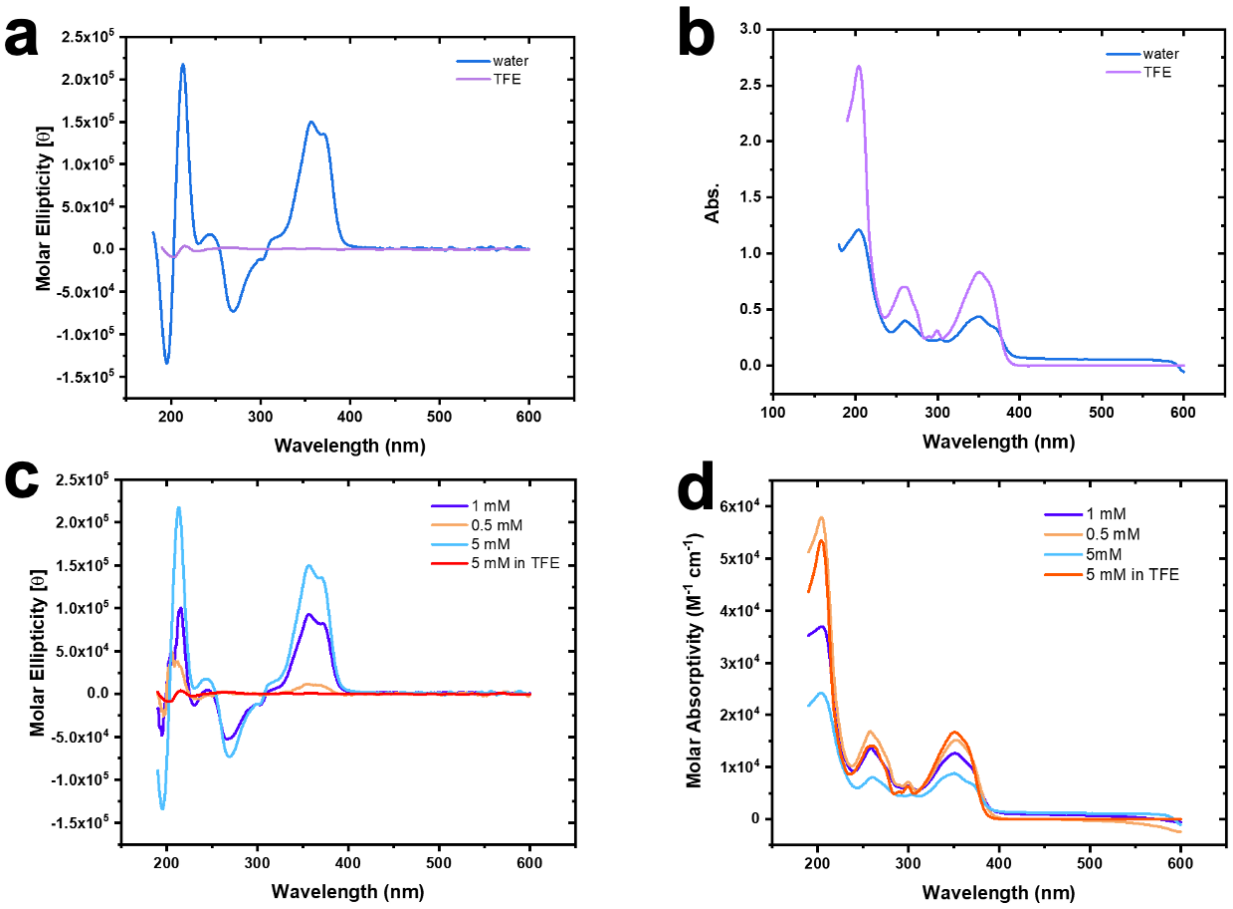


Figure S9. (a,c) CD and (b,d) UV-Vis spectra of **2** aged in (a,b) TFE or water at 5 mM (pH 7.0) for 7 days; and (c,d) as a function of concentration in water after 7 days, co-plotted with the monomeric state (dissolved in TFE). The Cotton effect at 350 nm in the CD spectra emerged from a π–π* absorption of the MC chromophore, indicating intermolecular *J*-type stacking interactions of adjacent MC groups in the self-assembled nanofibers.

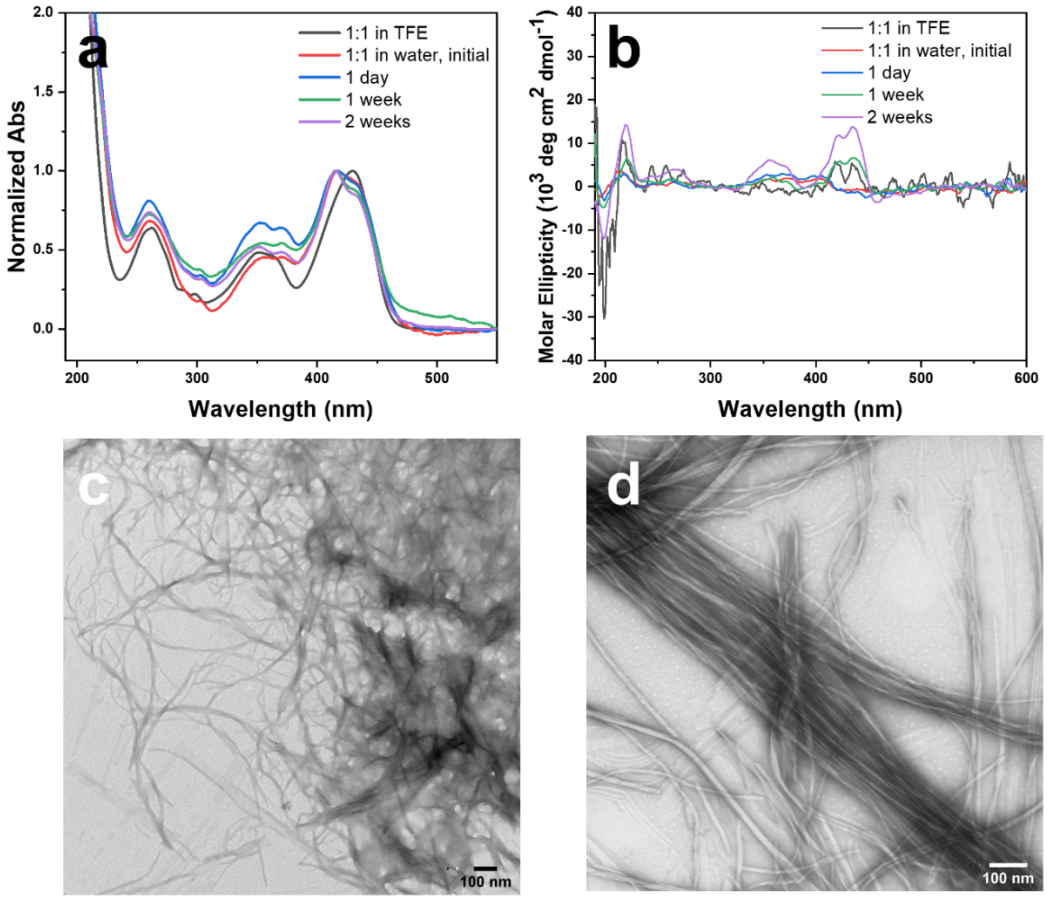


Figure S10. (a) UV-vis absorbance and (b) circular dichroism (CD) spectra of a mixture of 1 mM **1** and 1 mM **2** in a 1:1 molar ratio, each starting from monomeric states at pH 4.0. The mixtures were first dissolved in TFE to ensure a disassembled starting state, dried by lyophilization to remove solvent, then suspended in water. UV-vis and CD spectra were acquired with time points taken immediately after mixing, after 24 h, 1 week, and 2 weeks in a 1 mm quartz cuvette. Molar ellipticity was calculated with respect to **1**. Representative TEM images of the mixture were taken (c) 1 day after mixing and (d) after 2 weeks. Samples were prepared for TEM analysis by dropping the mixture onto carbon-coated copper grids for 1 min. After removal of excess solution, the sample grid was negatively stained with 2% (w/w) uranyl acetate solution for 30 s

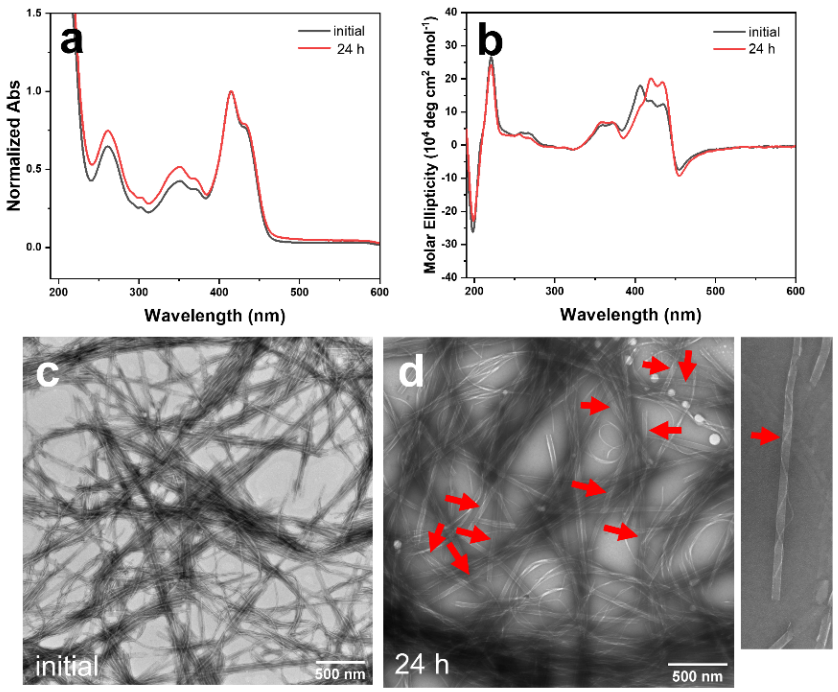


Figure S11. (a) UV-vis absorbance and (b) circular dichroism spectra of a mixture of pre-assembled 1 mM **1** and 1 mM **2** in a 1:1 molar ratio at pH 4.0 with time points taken immediately after mixing and after 24 h, in a 1 mm quartz cuvette. Molar ellipticity was calculated with respect to **1**. Representative TEM images of the mixture were taken (c) right after mixing and (d) after 24 h. The red arrows on the images indicate areas where it appeared that the fibers were integrated around the nanotubes. Samples were prepared for TEM analysis by dropping the mixture onto carbon-coated copper grids for 1 min. After removal of excess solution, the sample grid was negatively stained with 2% (w/w) uranyl acetate solution for 30 s

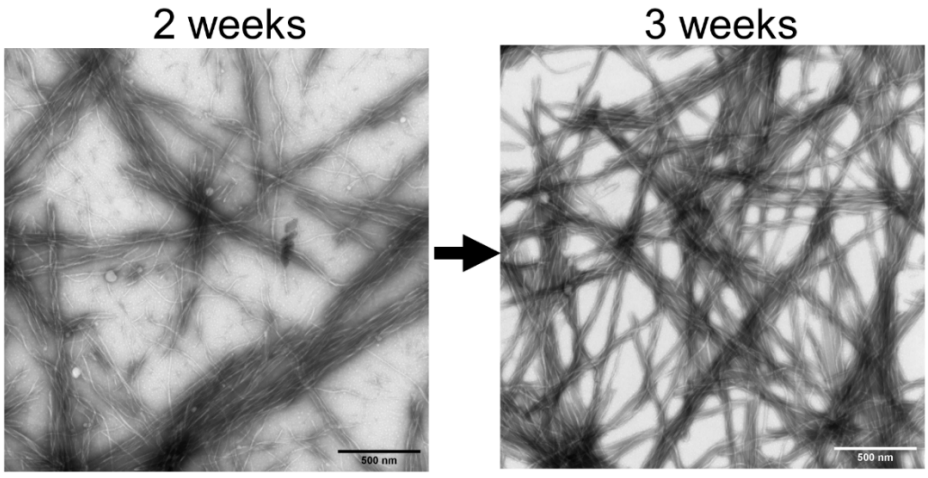


Figure S12. Representative TEM images of pre-assembled nanotubes of **1** (1 mM, pH 4.0) added to solid monomeric **2** (1 mM, prepared by first dissolving in TFE to disassemble any aggregates then freeze-drying) in a 1:1 molar ratio after 2 and 3 weeks. Samples were prepared for TEM analysis by dropping the mixture onto carbon-coated copper grids for 1 min. After removal of excess solution, the sample grid was negatively stained with 2% (w/w) uranyl acetate solution for 30 s.

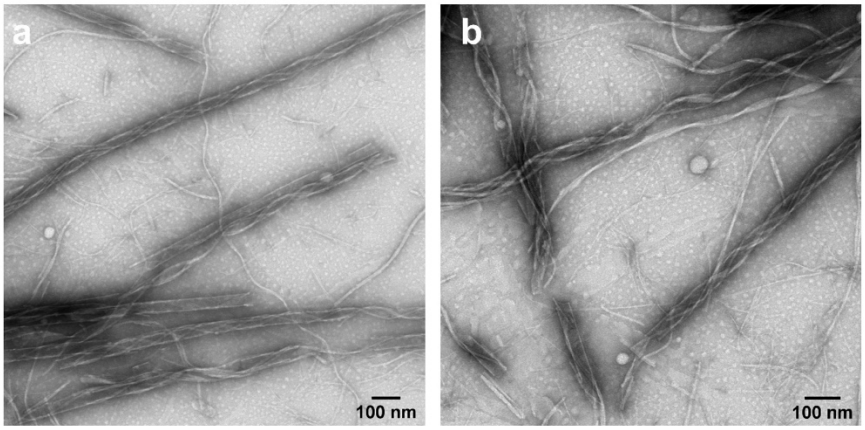


Figure S13. Regions of the TEM images showing partial unwinding of nanotubes. Samples of pre-assembled nanotubes of **1** (1 mM, pH 4.0) added to solid monomeric **2** (1 mM, prepared by first dissolving in TFE to disassemble any aggregates then freeze-drying) in a 1:1 molar ratio aged 2 weeks. Samples were prepared for TEM analysis by dropping the mixture onto carbon-coated copper grids for 1 min. After removal of excess solution, the sample grid was negatively stained with 2% (w/w) uranyl acetate solution for 30 s.

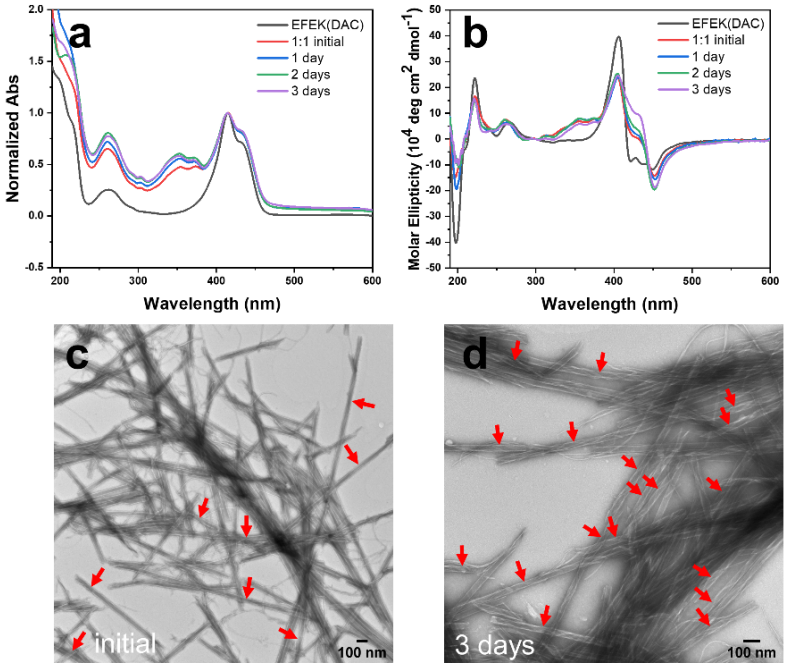


Figure S14. (a) UV-vis absorbance and (b) circular dichroism (CD) spectra of a mixture of 0.5 mM **1** assembled into nanotubes at pH 4.0 in a 1:1 molar ratio with **2** added as a monomeric solid (1mM, prepared by dissolving in TFE to disassemble any aggregates then freeze-drying), with selected time points over a timescale of 3 days. Measurements were taken in a 1 mm quartz cuvette. Molar ellipticity was calculated relative to **1**. Representative TEM images of the mixture were taken (c) immediately after mixing and (d) after 3 days. Red arrows indicated instances where fibers were visible wrapping around nanotube structures. Samples were prepared for TEM analysis by dropping the mixture onto carbon-coated copper grids for 1 min. After removal of excess solution, the sample grid was negatively stained with 2% (w/w) uranyl acetate solution for 30 s

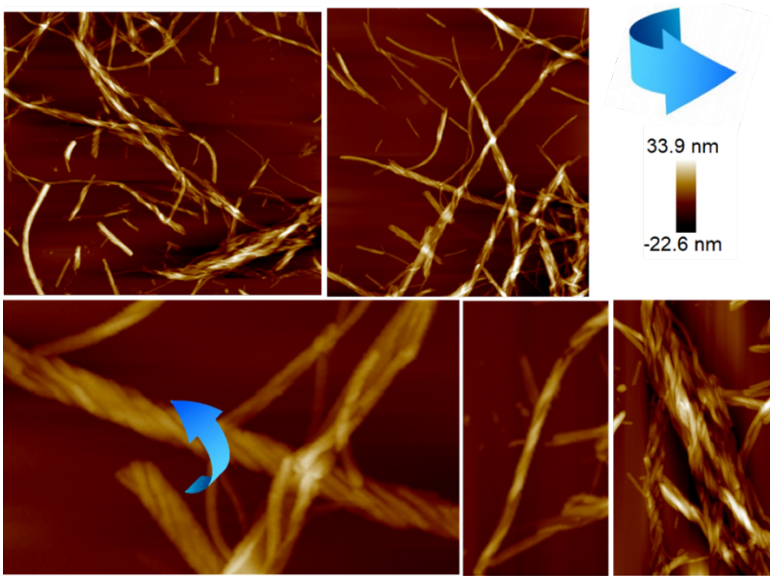


Figure S15. Samples of pre-assembled nanotubes of **1** (1 mM, pH 4.0) added to solid monomeric **2** (1 mM, prepared by first dissolving in TFE to disassemble any aggregates then freeze-drying) in a 1:1 molar ratio at pH 4.0, then aged for 3 weeks. Sample (1 mM) was deposited onto freshly cleaved mica for 1 min, rinsed with water for 10s, then dried with nitrogen gas.

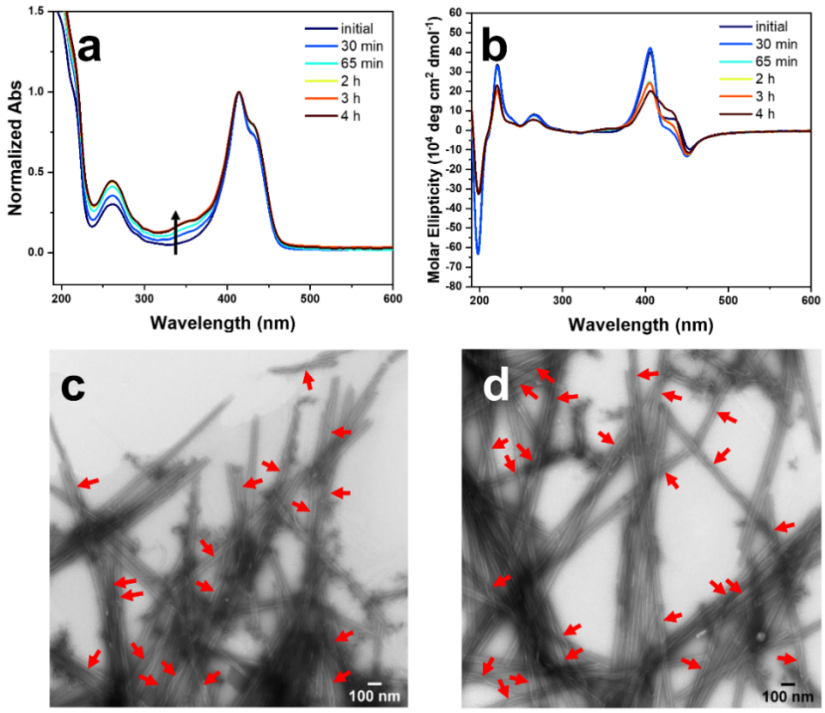


Figure S16. 1 mM solid at 30 °C (a) UV-vis absorbance and (b) circular dichroism (CD) spectra of a mixture of 1 mM **1** assembled into nanotubes at pH 4.0 in a 1:1 molar ratio with **2** added as a monomeric solid (1 mM, prepared by dissolving in TFE to disassemble any aggregates then freeze-drying), heated to 30 °C for 4 h. Samples were diluted to 250 μM **1** prior to measurement using a 1 mm path length cuvette. Molar ellipticity was calculated relative to **1**. Representative TEM images of the mixture (c) and (d) were taken after 4 h. Red arrows indicated instances where fibers were visible wrapping around nanotube structures.

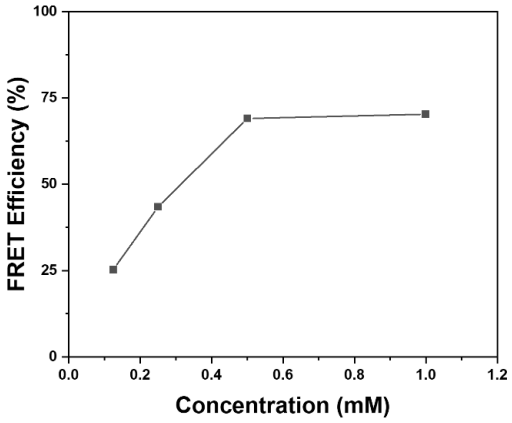


Figure S17. Pre-assembled **1** (1 mM) and pre-assembled **2** (1 mM) mixed at pH 6.0, then diluted in water to different concentrations. Samples were excited at 330 nm, using a triangle quartz cuvette. The spectra of **1** and **2** individually were taken at the same concentration and pH as the mixtures for comparison. FRET efficiencies (shown as a percent) were calculated at 415 nm, taking an average intensity value from 3 measurements per sample.

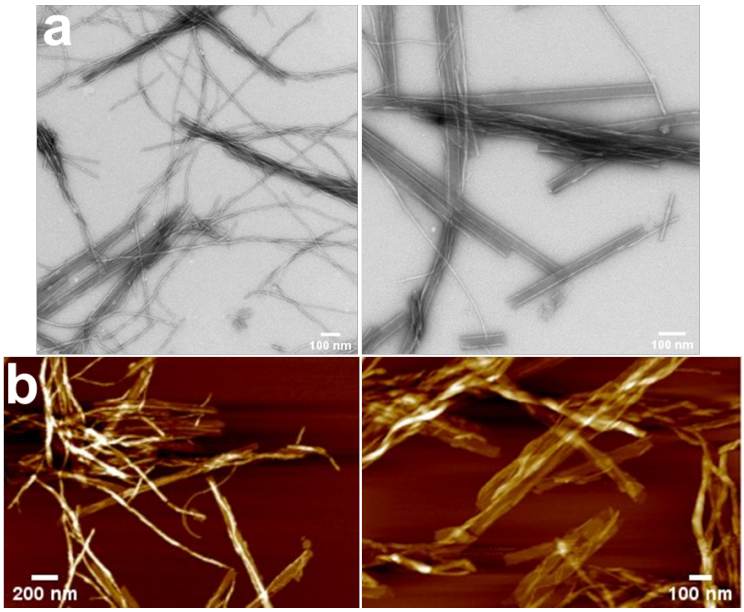


Figure S18. Co-assembly of **1** and **2** performed at 1.5 mM. Pre-assembled nanotubes of **1** (1.5 mM, pH 4.0) added to solid, monomeric **2** (1.5 mM, prepared by first dissolving in TFE to disassemble any aggregates then freeze-drying) in a 1:1 molar ratio at pH 4. Samples were imaged after three days at 1.5 mM by (a) TEM and (b) AFM. Samples for AFM were deposited onto freshly cleaved mica for 1 min, rinsed with water for 10 s, then dried with nitrogen gas. Samples were prepared for TEM analysis by dropping the mixture onto carbon-coated copper grids for 1 min. After removal of excess solution, the sample grid was negatively stained with 2% (w/w) uranyl acetate solution for 30 s.

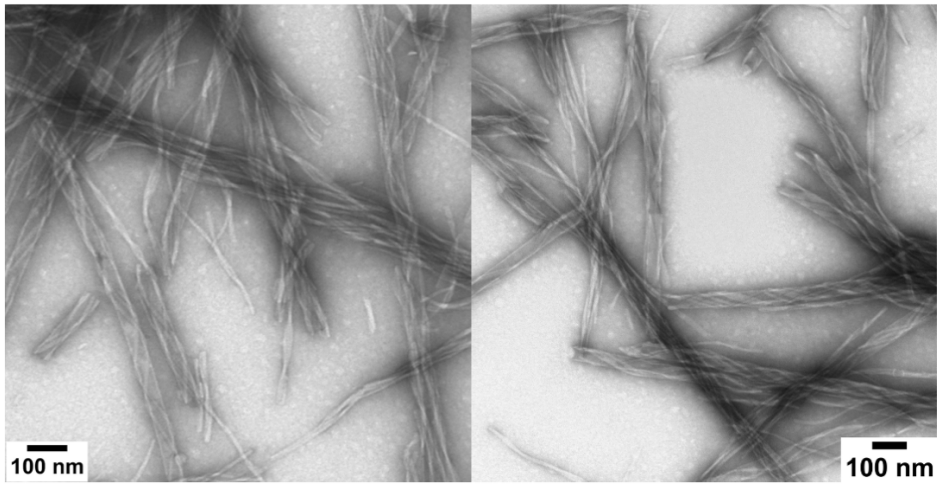


Figure S19. Representative TEM images of nanofiber-wrapped nanotubes after 9 months. Samples of pre-assembled nanotubes of **1** (1 mM, pH 4.0) added to solid monomeric **2** (1 mM, prepared by first dissolving in TFE to disassemble any aggregates then freeze-drying) in a 1:1 molar ratio. TEM images were taken after 9 months. Samples were prepared for TEM analysis by dropping the mixture onto carbon-coated copper grids for 1 min. After removal of excess solution, the sample grid was negatively stained with 2% (w/w) uranyl acetate solution for 30 s.

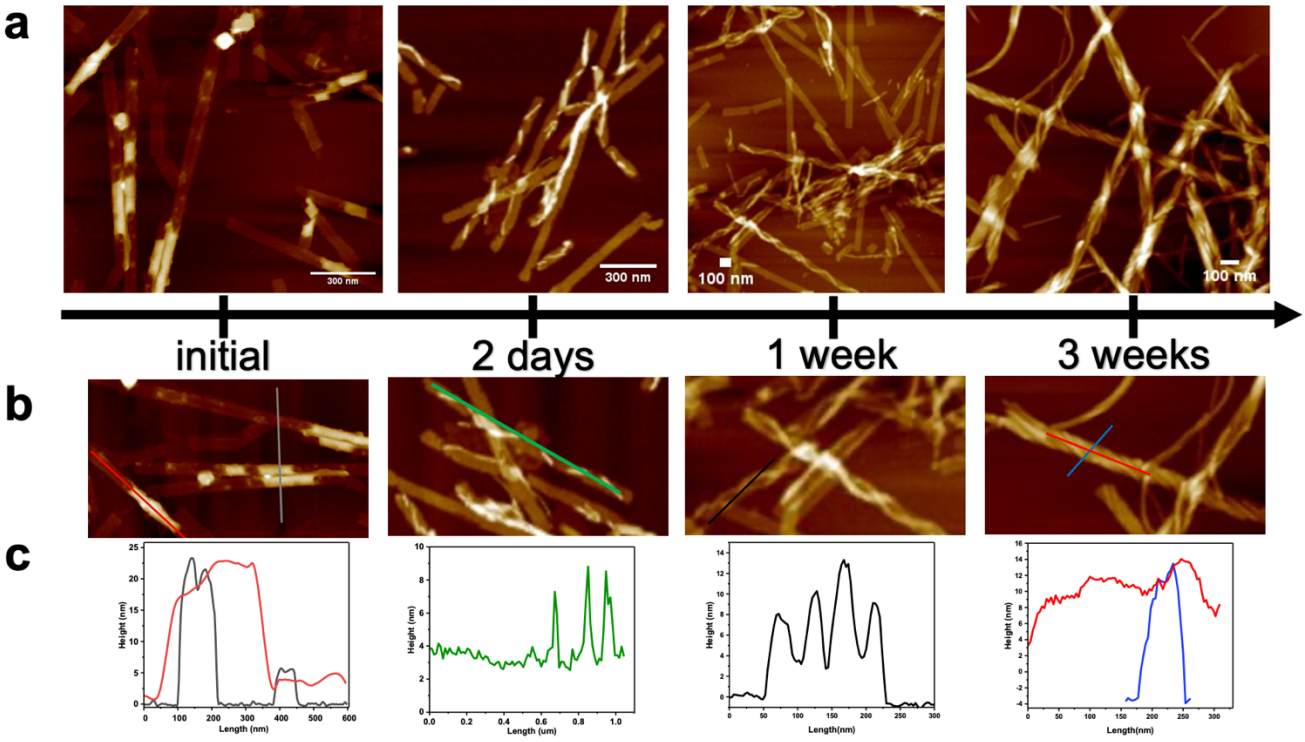


Figure S20. Samples of pre-assembled nanotubes of **1** (1 mM, pH 4.0) added to solid monomeric **2** (1 mM, prepared by first dissolving in TFE to disassemble any aggregates then freeze-drying) in a 1:1 molar ratio. (a) representative AFM images of the fiber-wrapped nanotubes at various time points as labeled (b) zoomed in nanotubes for better visualization with marked with colored lines that correlate with the height profiles measured in (c). (c) Plots of AFM cross-sectional height profiles shown above in row (b). Samples for AFM were prepared by depositing onto freshly cleaved mica for 1 min, rinsing with water for 10 s, then dried with nitrogen gas.

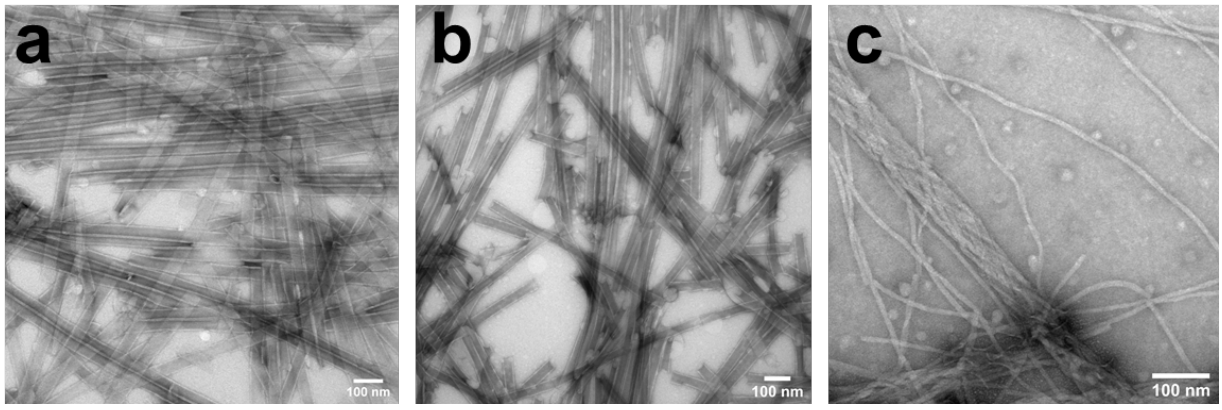


Figure S21. TEM images of co-assembly after 3 days at varying **1:2** ratios. Pre-assembled nanotubes of **1** (1.0 mM, pH 4.0) added to monomeric **2** (1.0 mM, prepared by first dissolving in TFE to disassemble any aggregates then freeze-drying) at various **1**:**2** ratios at pH 4.0: (a) 10:1 (b) 5:1 (c) 1:5 ratio. Samples were prepared for TEM analysis by dropping the mixture onto carbon-coated copper grids for 1 min. After removal of excess solution, the sample grid was negatively stained with 2% (w/w) uranyl acetate solution for 30 s.

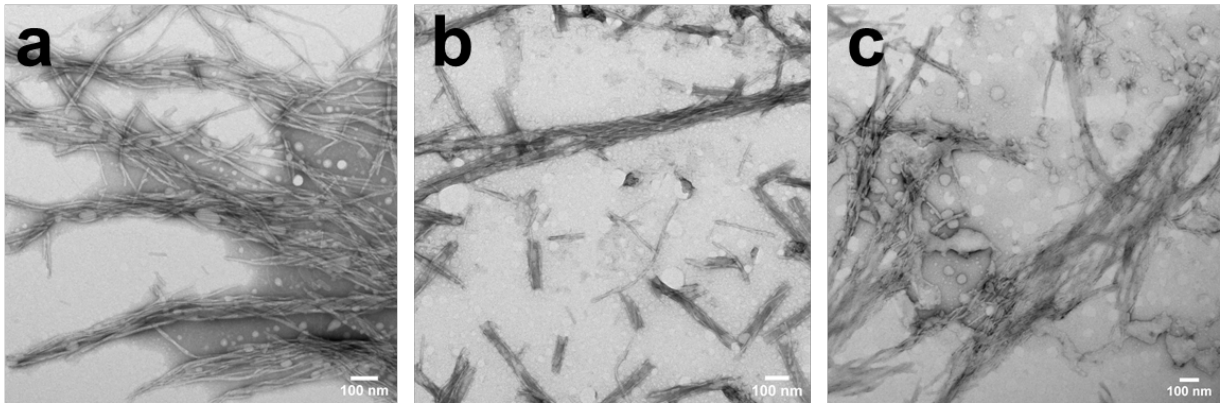


Figure S22. TEM images of 1:1 co-assembly after 3 days and dilution. Pre-assembled nanotubes of **1** (1.0 mM, pH 4.0) added to monomeric **2** (1.0 mM, prepared by first dissolving in TFE to disassemble any aggregates then freeze-drying) in a 1:1 ratio. Aged at least three weeks and diluted to (a) 0.5 mM (b) 0.25 mM (c) 0.125 mM. Samples were prepared for TEM analysis by dropping the mixture onto carbon-coated copper grids for 1 min. After removal of excess solution, the sample grid was negatively stained with 2% (w/w) uranyl acetate solution for 30 s.

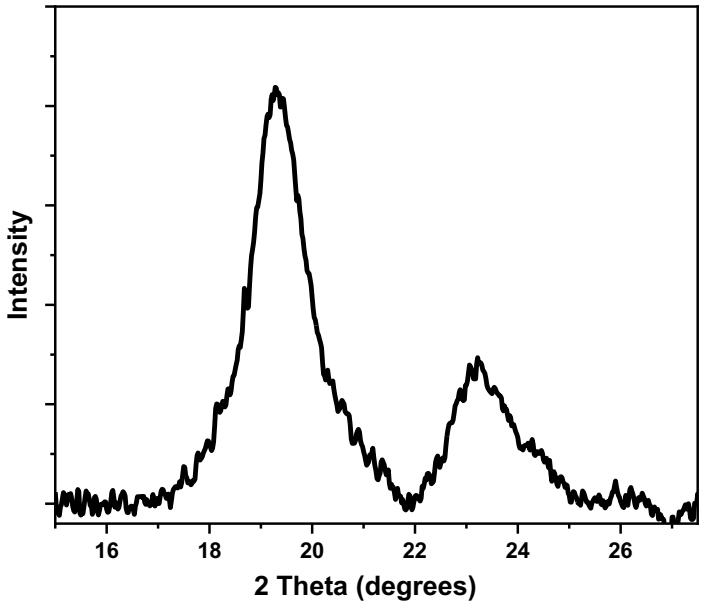


Figure S23. Samples of pre-assembled nanotubes of **1** (1 mM, pH 4.0) added to solid monomeric **2** (1 mM, prepared by first dissolving in TFE to disassemble any aggregates then freeze-drying) in a 1:1 molar ratio at pH 4 and assembled into fiber wrapped nanotubes over 3 weeks. Fiber wrapped nanotubes were lyophilized to obtain solid powder for the XRD measurement. The 2θ peak maximums were found at 19.3° and 23.1° which were calculated to find d spacing values of 4.594 Å and 3.850 Å.

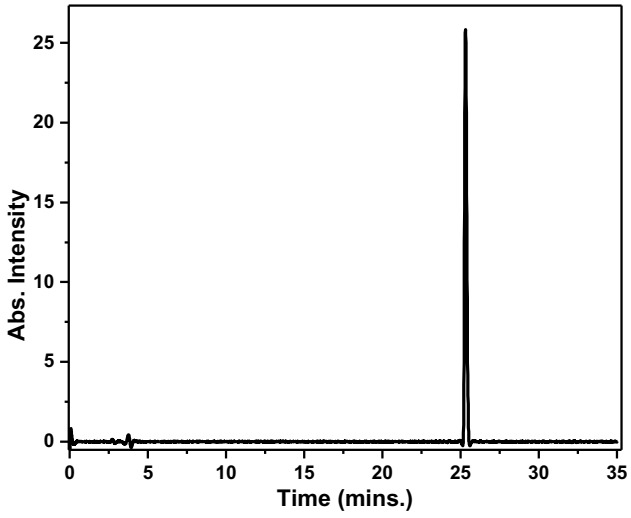


Figure S24. Analytical HPLC trace (1 mL/min, ramped from 20-60% CH3CN in water) of **2** after purification via preparatory HPLC.

NMR Spectra:

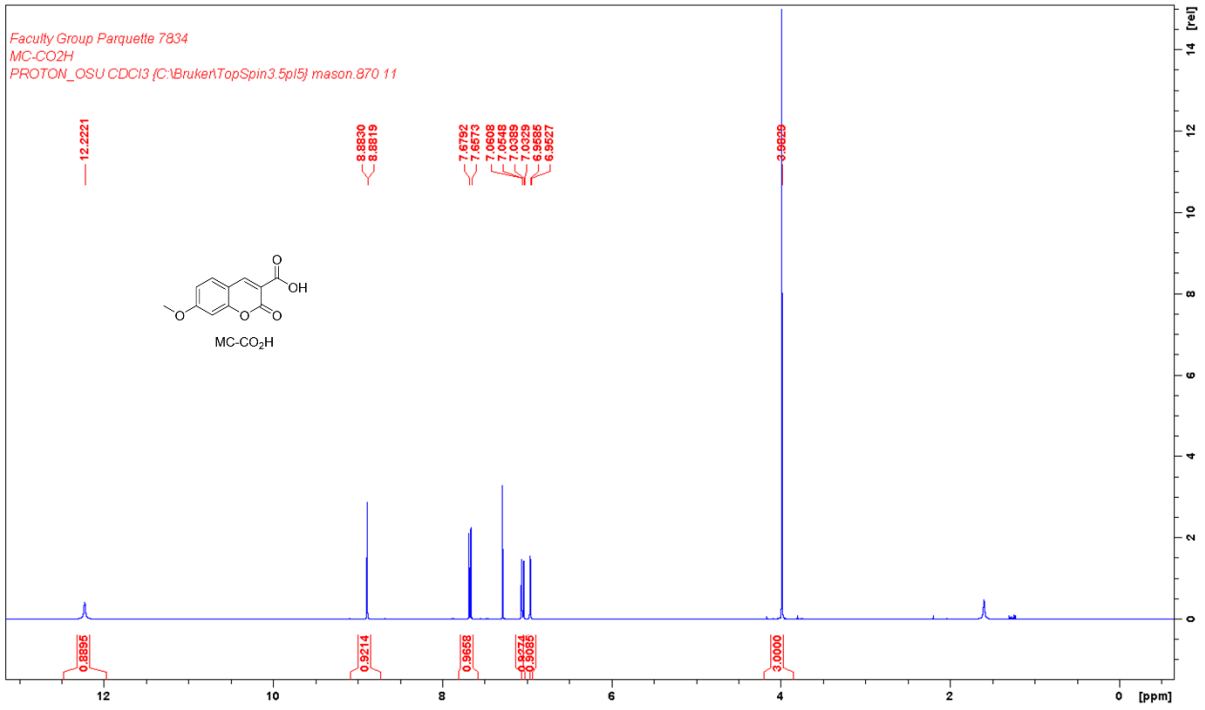


Figure S25. ^{1}H NMR spectrum of MC-CO_2H in $CDCl_3$.

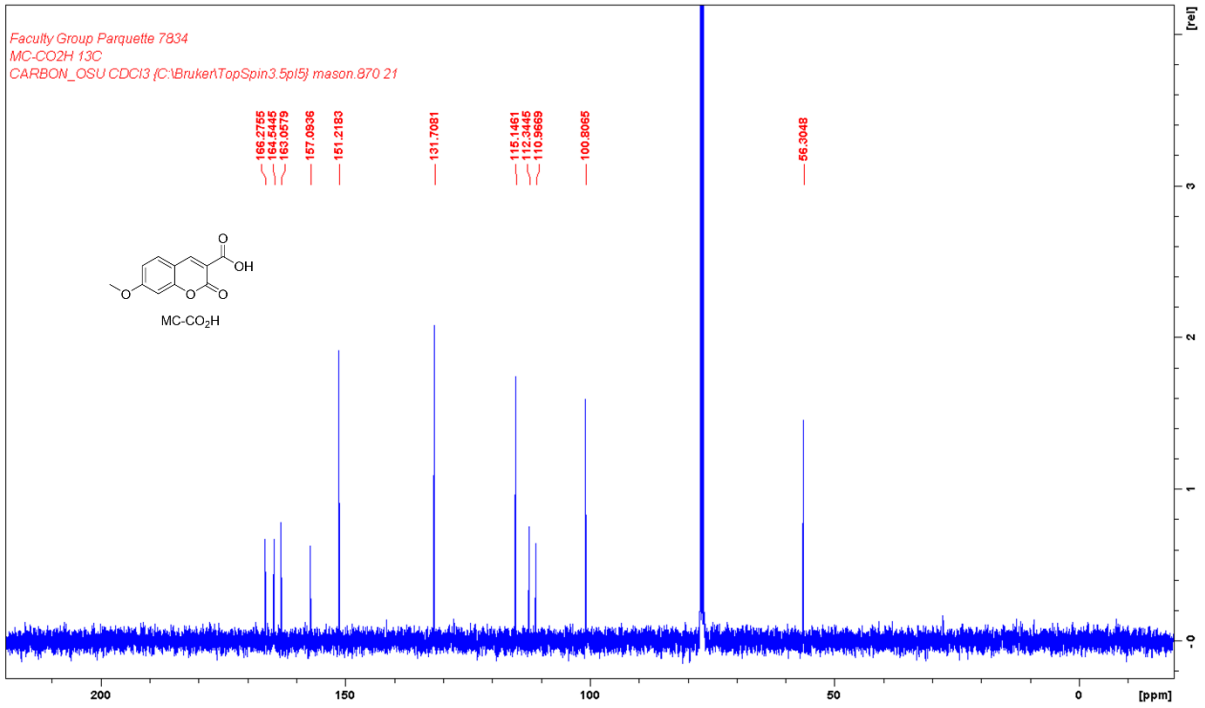


Figure S26. ^{13}C NMR spectrum of MC-CO_2H in $CDCl_3$.

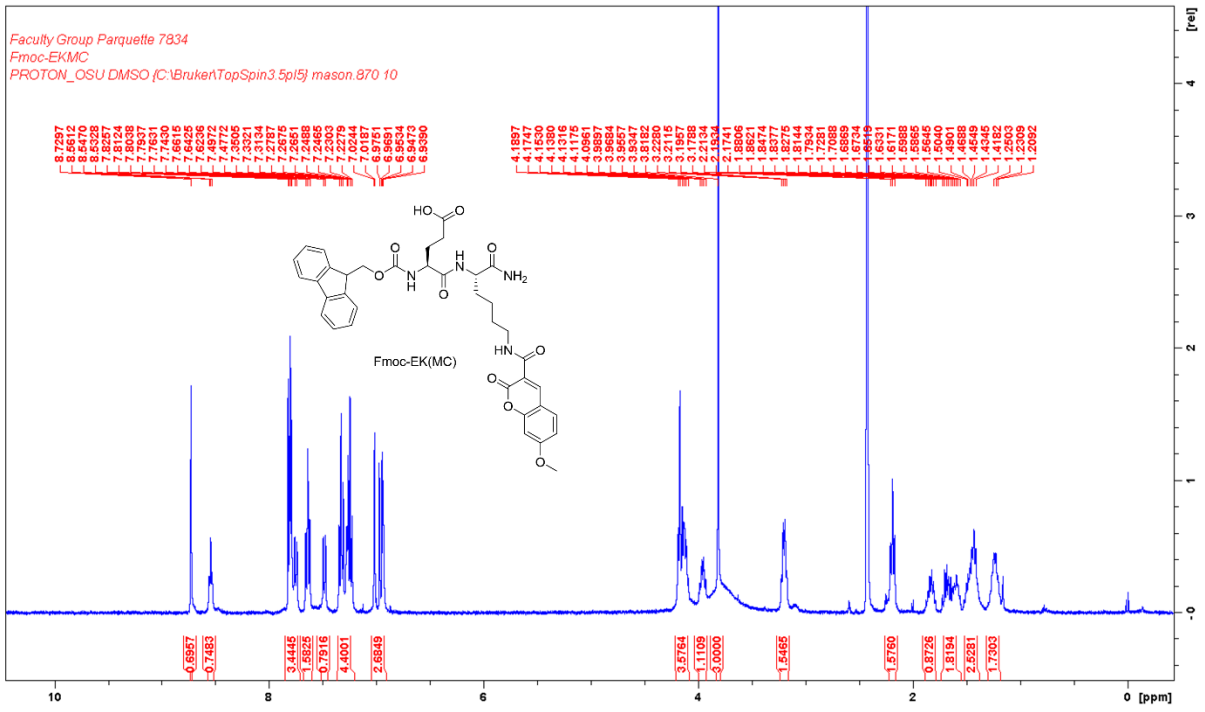


Figure S27. ^{1}H NMR spectrum of Fmoc-EK(MC) in DMSO-d6.

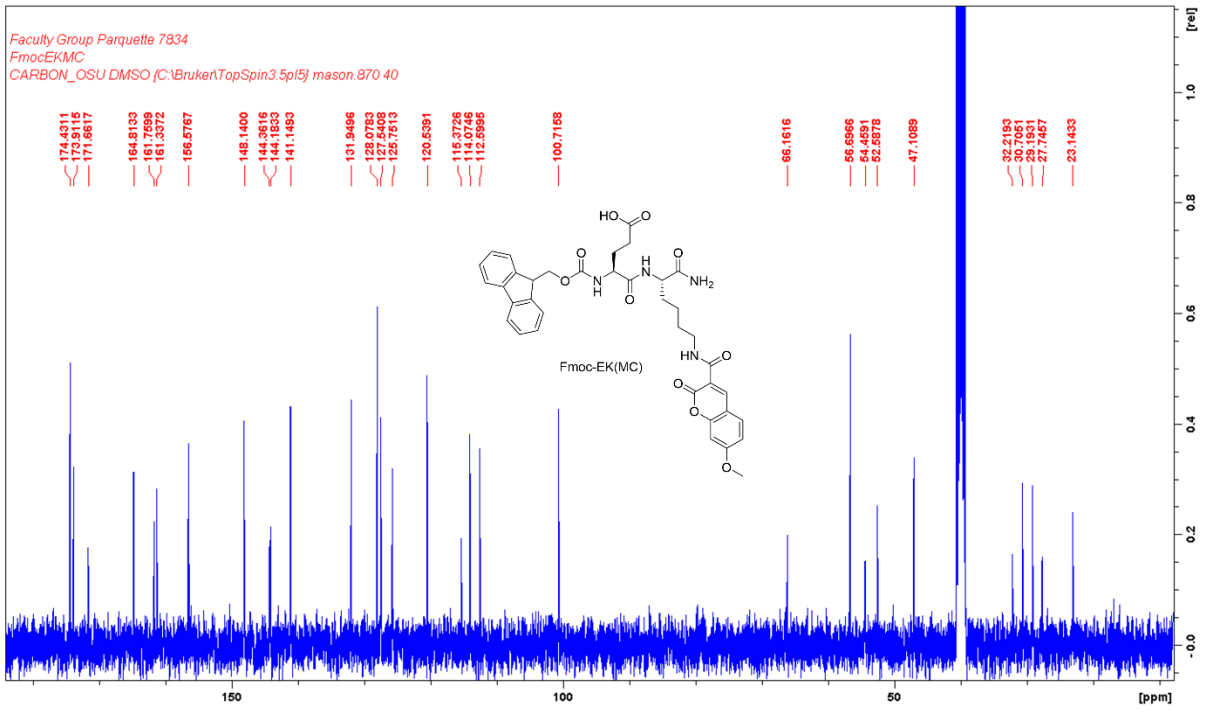


Figure S28. ^{13}C NMR spectrum of Fmoc-EK(MC) in DMSO-d6.

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