

Supporting Information

Programmable Assembly of Peptide Amphiphile via Noncovalent-to-Covalent Bond Conversion

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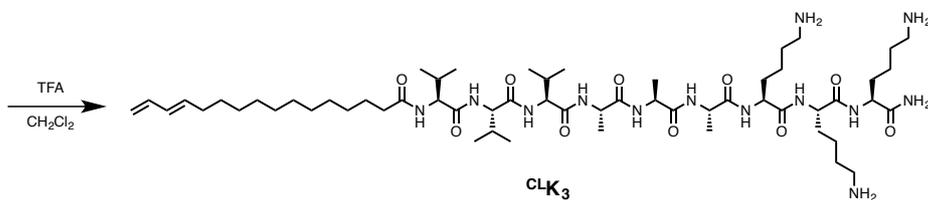
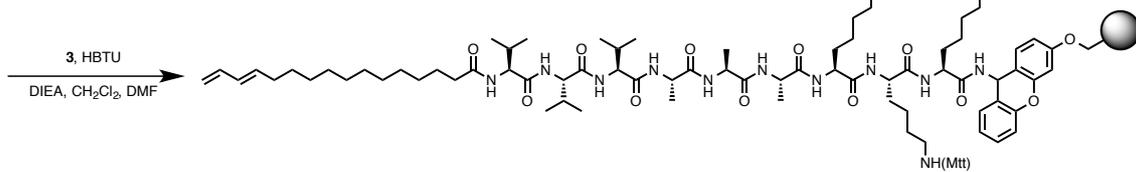
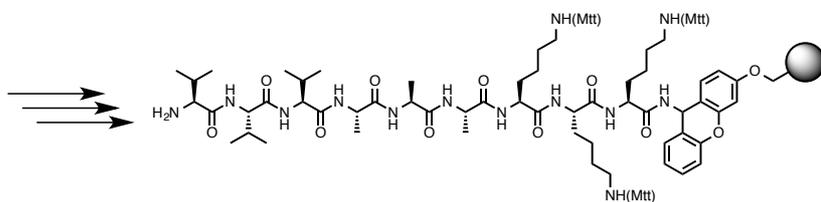
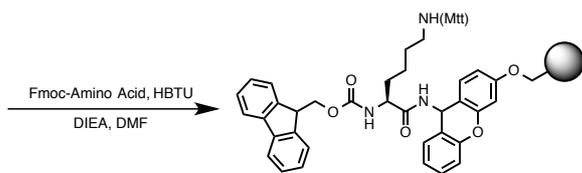
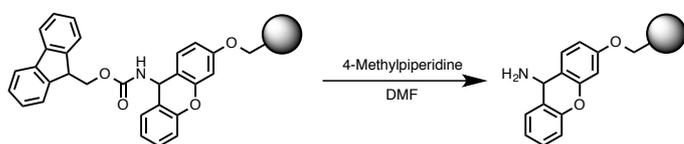
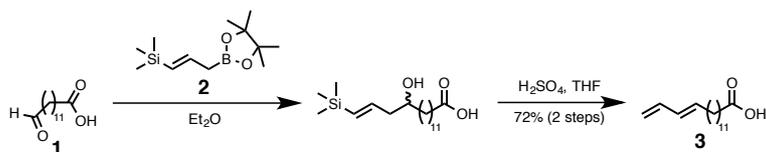
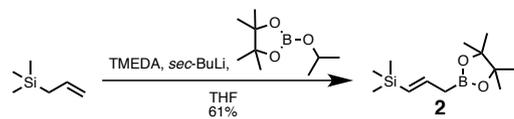
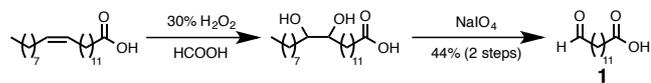
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1. General

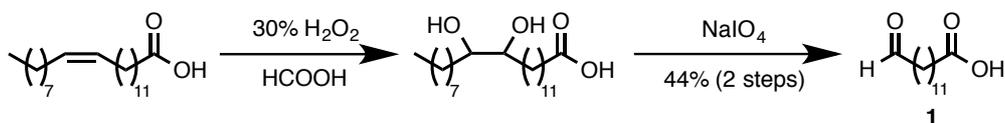
Unless otherwise noted, all commercial reagents were used as received. For column chromatography, Silica gel 60 (particle size 60–200 μm ; silica; Alfa Aesar) was used. Preparative reverse-phase high-performance liquid chromatography (RP-HPLC) was performed at 25 °C using a Phenomenex Kinetex column (C_{18} stationary phase, 5 μm , 100 Å pore size, 30.0 \times 150 mm) on a Shimadzu model prominence modular HPLC system equipped with a DGU-20A_{5R} degassing unit, two LC-20AP solvent delivery units, a SPD-M20A diode array detector and a FRC-10A fraction collector, using $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ gradient containing 0.1% CF_3COOH (v/v) as an eluent at a flow rate of 25.0 mL min^{-1} . Analytical RP-HPLC was performed at 25 °C using a Phenomenex Jupiter 4U Proteo column (C_{12} stationary phase, 4 μm , 90 Å pore size, 4.60 \times 150 mm) on an Agilent model 1260 Infinity II LC system, using $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ gradient containing 0.1% CF_3COOH (v/v) as an eluent at a flow rate of 1.0 mL min^{-1} . SEC (Size exclusion chromatography) in hexafluoroisopropanol (HFIP) was performed with 3 g/L potassium trifluoroacetate at 40 °C. The columns were packed with modified silica (PFG columns, particle size: 7 μm , porosity: 100 and 1000 Å). A refractive index detector (G 1362A RID, Jasco) and a UV/vis detector (UV-2075 Plus, JASCO) were used to detect the polymer. Molecular weights were calculated using calibration performed with PMMA standards (Polymer Standards Services GmbH). ^1H and ^{13}C NMR spectra were recorded on a Varian model Inova 500 spectrometer, operating at 500 and 125 MHz for ^1H and ^{13}C NMR, respectively, where chemical shifts were determined with respect to tetramethylsilane as an internal reference. Fourier-transform infrared (FT-IR) spectra were recorded on a Bruker model Tensor 37 Fourier-transform infrared spectrometer using an ATR attachment. Electrospray ionization mass (ESI-mass) spectrometry was performed in positive scan mode on an Agilent model 6510 Quadrupole Time-of-Flight LC/MS spectrometer using direct injection. Matrix-assisted laser deposition ionization time-of-flight (MALDI-TOF) mass spectrometry was performed in the linear mode on a Bruker autoflex III smartbeam spectrometer. Circular dichroism (CD) spectra were recorded on a JASCO model J-815 spectropolarimeter using a quartz cell of 0.1 mm optical path length. Electronic absorption spectra were recorded on a PerkinElmer model Lambda 1050 UV/Vis/NIR spectrophotometer using a quartz cell of 0.1 mm optical path length. Dynamic light

scattering (DLS) measurements were performed by a Malven model Zetasizer Nano ZSP light scattering spectrometer. Fluorescent spectra were recorded on a JASCO model J-815 spectropolarimeter using a low volume quartz cell of 10 mm optical path length. Transmission electron microscopy (TEM) images were obtained using a Hitachi model HT-7700 electron microscope operating at 80 kV, equipped with an Orius SC 1000A camera. Plunge-freezing for cryogenic TEM samples were performed using a FEI model Vitrobot Mark IV. Differential scanning calorimetry (DSC) thermograms were obtained using a GE Healthcare model MicroCal VP-DSC with medium feedback gain and filtering period of 1 s. Heating and cooling cycles were performed at 1 °C min⁻¹ from 20 °C to 60 °C. For cell studies, Mouse myoblast C2C12 (CRL-1772™) and Dulbecco's modified Eagle's medium (DMEM) (Catalog No. 30-2002) were purchased from ATCC. HyClone™ Fetal Bovine Serum (Catalog No. SH30071.031IR) was purchased from GE Healthcare Life Sciences. Dulbecco's phosphate-buffered saline (DPBS, Catalog No. 14190144), penicillin/streptomycin (Catalog No. 15140122), Trypsin-EDTA (Catalog No. 25200056), and LIVE/DEAD® Viability/Cytotoxicity Kit for mammalian cells (Catalog No. L3224) were obtained from ThermoFisher Scientific.

2. Synthesis



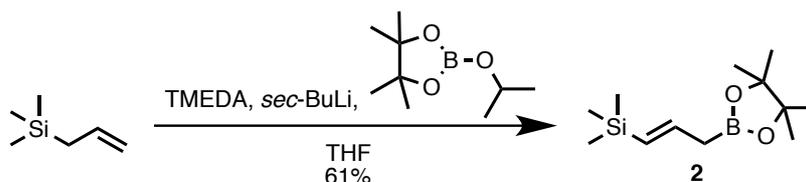
2.1. Synthesis of 1



Compound **1** was synthesized according to a method analogous to that reported previously.^{S1} To a H₂O₂ suspension (30% w/w in H₂O, 10 mL) of erucic acid (20 g, 60 mmol) was added formic acid (36 mL) at room temperature, and the resultant mixture was stirred at 55 °C for 1.5 hours. Then, the reaction mixture was cooled to room temperature and stirred for 22 hours. Formic acid was evaporated under reduced pressure, and aqueous NaOH (12.5 M, 40 mL) was slowly added at 0 °C. After stirring for 2.5 hours at room temperature, the mixture was cooled to 0 °C, and aqueous HCl (12 M, 20 mL) was slowly added to acidify to pH 1. The resultant precipitate was filtered and washed with H₂O, dried under reduced pressure, affording diol as a white solid (22 g), which was used in the next step without further purification.

To a CHCl₃ suspension (160 mL) of diol (22 g) was added aqueous NaIO₄ (0.5 M, 480 mL) at room temperature, and the resultant mixture was stirred for 30 hours at the same temperature. The reaction mixture was extracted with CHCl₃, washed successively with water and brine, and the combined organic extract was dried over Na₂SO₄ and evaporated to dryness under reduced pressure. The residue was subjected to column chromatography on silica gel using *n*-hexane/Et₂O/AcOH (100/50/1 v/v/v) as an eluent to allow isolation of compound **1** as a white solid (6.1 g, 27 mmol, 45%). ¹H NMR (500 MHz, CDCl₃, 25 °C, ppm) δ 9.72 (s, 1H), 2.38 (td, *J* = 7.4, 1.8 Hz, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 1.59 (m, 4H), 1.33–1.20 (m, 14H); ¹³C NMR (125 MHz, CDCl₃, 25 °C, ppm) δ 203.04, 180.12, 43.81, 34.03, 29.41, 29.31, 29.27, 29.15, 29.09, 28.98, 24.61, 22.01.

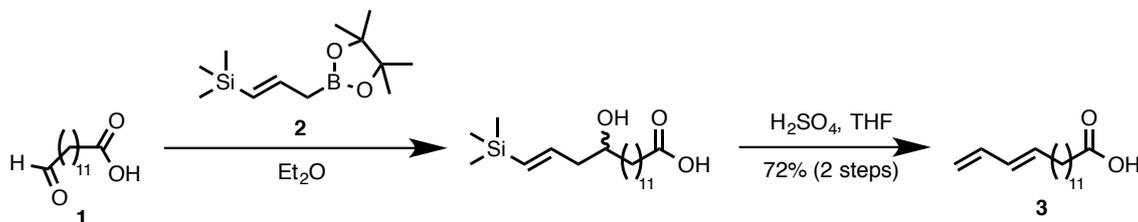
2.2. Synthesis of 2



Compound **2** (Matteson's reagent) was synthesized according to a method

analogous to that reported previously.^{S2,33} To a THF solution (80 mL) of tetramethylethylenediamine (TMEDA, 19.6 mL, 130mmol) and *sec*-butyl lithium (1.4 M in cyclohexane, 93 mL, 130 mmol) was added allyltrimethylsilane (20.7 mL, 130 mmol) dropwisely under N₂ at -78 °C, and the resultant mixture was stirred for 2 hours at -40 °C. Then, the reaction mixture was transferred to a THF solution (60 mL) of 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (26.3 mL, 130 mmol) under N₂ at -78 °C. The reaction mixture was warmed to room temperature and stirred for 14 hours. To this reaction mixture was added CH₂Cl₂ (300 mL), saturated aqueous NH₄Cl (150 mL) and aqueous HCl (1 M, 150 mL). The mixture was extracted with Et₂O, washed successively with with water and brine, and the combined organic extract was dried over Na₂SO₄ and evaporated to dryness under reduced pressure. The residue was subjected to column chromatography on silica gel using *n*-hexane/Et₂O (25/1 v/v) as an eluent to allow isolation of compound **2** as a clear oil (18.9 g, 78.8 mmol, 61%). ¹H NMR (500 MHz, CDCl₃, 25 °C, ppm) δ 6.01 (dt, *J* = 18.4, 7.1 Hz, 1H), 5.56 (dt, *J* = 18.4, 1.5 Hz, 1H), 1.75 (d, *J* = 7.1 Hz, 2H), 1.20 (s, 12H), -0.01 (s, 9H); ¹³C NMR (125 MHz, CDCl₃, 25 °C, ppm) δ 142.01, 130.51, 83.03, 24.63, -1.15.

2.3. Synthesis of **3**



Compound **3** was synthesized according to a method analogous to that reported previously.³³ To a Et₂O solution (26 mL) of compound **2** (4.8 g, 20 mmol) was added compound **1** (4.6 g, 20 mmol) under N₂ at room temperature, and the resultant mixture was stirred for 96 hours at the same temperature. To this reaction mixture was added triethanolamine (5.8 mL, 44 mmol), and the resulting mixture was stirred for 22 hours at room temperature. Then, aqueous HCl (1M, 100 mL) was added, and the mixture was extracted with CH₂Cl₂ (100 mL, 2 times). The combined organic extract was dried over Na₂SO₄ and evaporated to dryness under reduced pressure. The residue was subjected to column chromatography on silica gel using CH₂Cl₂/MeOH (19/1 v/v) as an eluent to allow isolation of an intermediate carboxylic

acid as a white solid (7.1 g).

To a THF solution (7.5 mL) of carboxylic acid (3.43 g, 10 mmol) was added 2 drops of concentrated H₂SO₄ at room temperature, and the mixture was stirred for 23 hours at the same temperature under dark. Then, the reaction mixture was evaporated to dryness under reduced pressure. The residue was subjected to column chromatography on silica gel using *n*-hexane/Et₂O/AcOH (100/30/1 v/v/v) as an eluent to allow isolation of compound **3** as a white solid (1.82 g, 7.2 mmol, 72%). ¹H NMR (500 MHz, CDCl₃, 25 °C, ppm): δ 6.31 (dt, *J* = 17.0, 10.3 Hz, 1H), 6.04 (dd, *J* = 15.2, 10.4 Hz, 1H), 5.71 (dt, *J* = 15.2, 7.0 Hz, 1H), 5.06 (d, *J* = 16.6 Hz, 1H), 4.94 (d, *J* = 10.1 Hz, 1H), 2.34 (t, *J* = 7.5, 2H), 2.06 (m, 2H), 1.63 (m, 2H), 1.26 (m, 16H); ¹³C NMR (125 MHz, CDCl₃, 20 °C, ppm): δ 179.79, 137.38, 135.63, 130.84, 114.54, 34.00, 32.56, 29.55, 29.48, 29.42, 29.23, 29.21, 29.20, 29.06; ESI-MS *m/z* calcd. for C₁₆H₃₁NO₂ [M + NH₃]⁺: *m/z* = 269.24, found: 269.24.

2.4. Synthesis of peptide

Peptide was synthesized in a 2.0 mmol scale on Sieber resin (0.70 meq g⁻¹ 100–200 mesh) employing a standard Fmoc solid-phase peptide synthesis (SPPS) method.^{S3,S4} For peptide coupling, to a DMF solution (50 mL) of Fmoc-protected amino acid (8.0 mmol) and *N,N,N',N'*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uranium hexafluorophosphate (HBTU, 3 g, 7.9 mmol) was added *N,N*-diisopropylethylamine (DIPEA, 2.1 mL, 12 mmol), and the mixture was stirred for 1 min for activation. The mixture was then added to peptidyl resin, and the reaction vessel was shaken for 2 hours at 25 °C. For Fmoc deprotection, after the coupling solution was drained off, the peptidyl resin was washed with DMF (3 times), and 30% 4-methylpiperidine in DMF (v/v) was added to the peptidyl resin. After shaking for 10 min, the reaction solution was drained off. This reaction was repeated one more time, and the resulting peptidyl resin was washed with DMF (3 times) and CH₂Cl₂ (1 time).

2.5. Synthesis of ¹⁴C-**K**₃

To a DMF/CH₂Cl₂ (1/1 v/v) solution (30 mL) of compound **3** (757 mg, 3.0 mmol) and HBTU (1.14 g, 3.0 mmol) was added DIPEA (1.0 mL, 6.0 mmol), and the mixture was stirred for 1 min for activation. The mixture was then added to peptidyl resin, and the reaction vessel was shaken for 3 hours at 25 °C under dark. After the

reaction solution was drained off, the peptidyl resin was washed with DMF (3 times) and CH₂Cl₂ (3 times).

A cleavage reaction of peptide amphiphile from the peptidyl resin was performed twice in a 1.0 mmol scale. To a CH₂Cl₂ solution (150 mL) of trifluoroacetic acid (TFA, 3% v/v) was added the peptidyl resin (1.0 mmol). After shaking for 30 min, the cleavage mixture and two subsequent CH₂Cl₂ washings were filtered. The combined solution was evaporated to a viscous solution under reduced pressure. Then, cold Et₂O was added to the solution and the resulting precipitate was washed with cold Et₂O (3 times) to remove residual TFA. The residue was purified by RP-HPLC to allow isolation of ^{CL}**K**₃ as a white solid (354 mg, 0.238 mmol, 12%). The purified compound was stored at -20 °C under dark. ¹H NMR (500 MHz, DMSO-*d*₆, 25 °C, ppm): δ 7.80–8.02 (m, 8H), 7.65 (br, 10H), 7.30 (s, 1H), 7.04 (s, 1H), 6.29 (dt, *J* = 16.8, 10.5 Hz, 1H), 6.03 (dd, *J* = 15.1, 10.5 Hz, 1H), 5.72 (dt, *J* = 15.2, 7.0 Hz, 1H), 5.07 (d, *J* = 17.1 Hz, 1H), 4.94 (d, *J* = 9.9 Hz, 1H), 4.19 (m, 9H), 2.74 (br, 6H), 2.13 (m, 2H), 2.05 (m, 2H), 1.96 (m, 3H), 1.67 (m, 3H), 1.51 (m, 10H), 1.15–1.40 (m, 34H), 0.77–0.87 (m, 18H); ESI-MS *m/z* calcd. for C₅₈H₁₀₇N₁₃O₁₀ [M]⁺: *m/z* = 1145.83, found: 1145.73; analytical RP-HPLC: see Figure S5.

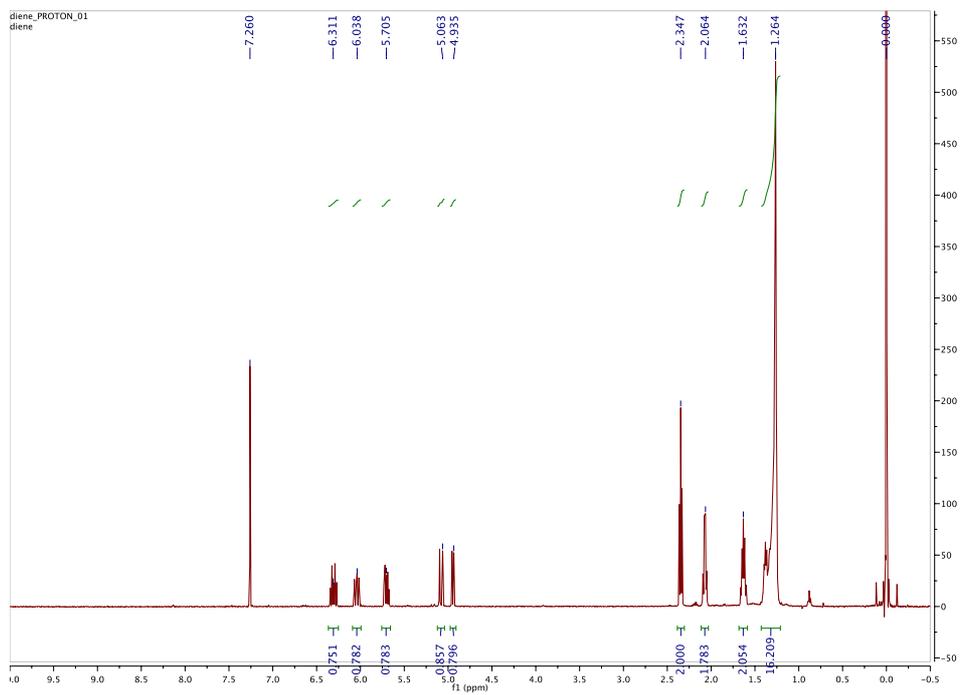


Figure S1. ^1H NMR spectrum of **3** in CDCl_3 at $25\text{ }^\circ\text{C}$.

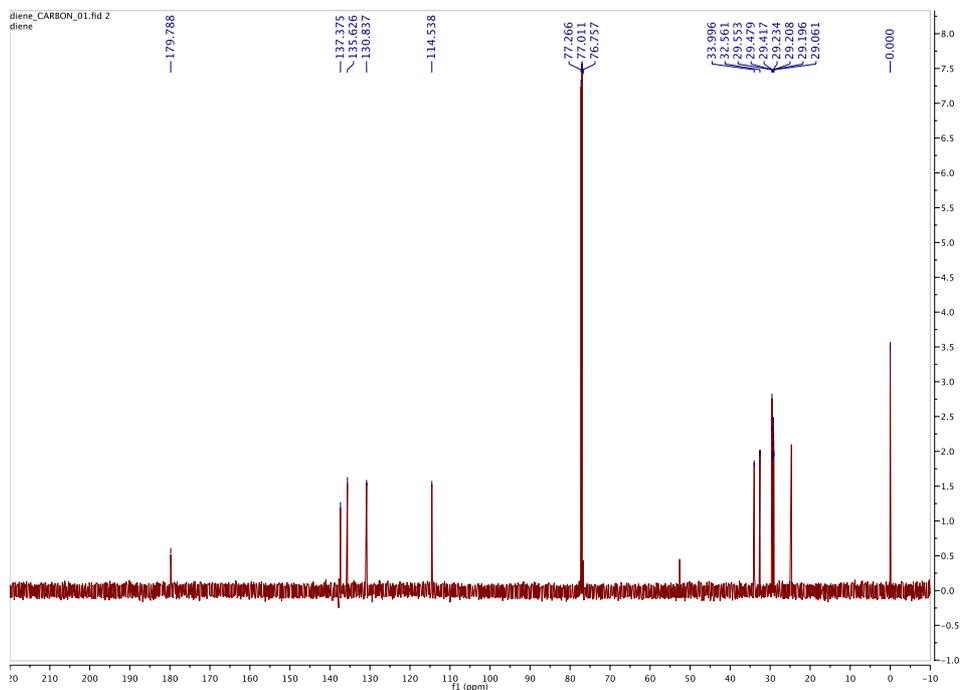


Figure S2. ^{13}C NMR spectrum of **3** in CDCl_3 at $25\text{ }^\circ\text{C}$.

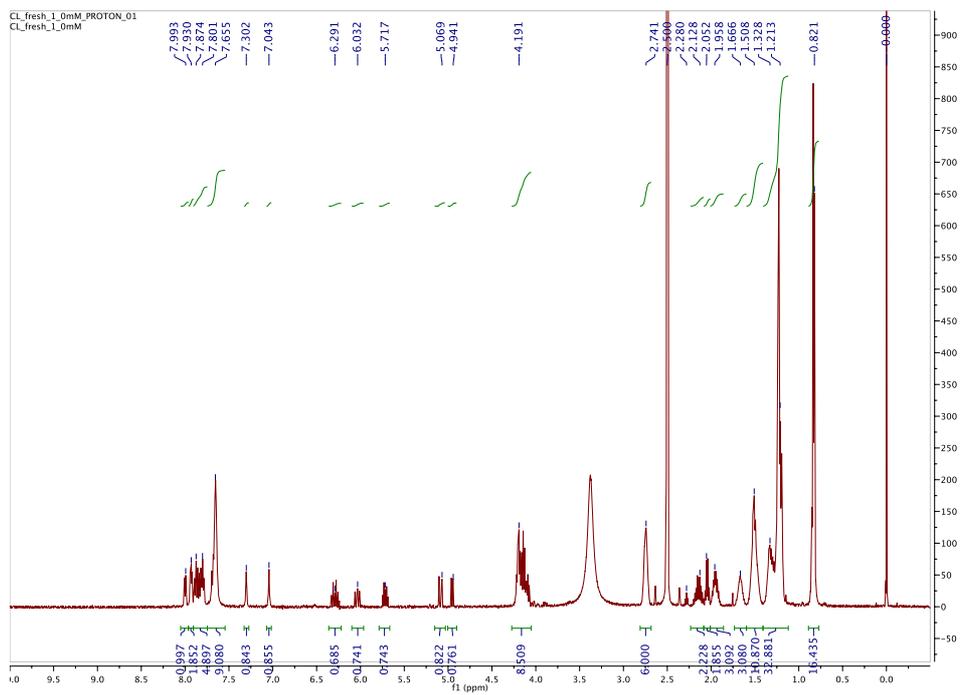


Figure S3. ^1H NMR spectrum of $^{\text{CL}}\text{K}_3$ in $\text{DMSO-}d_6$ at $25\text{ }^\circ\text{C}$.

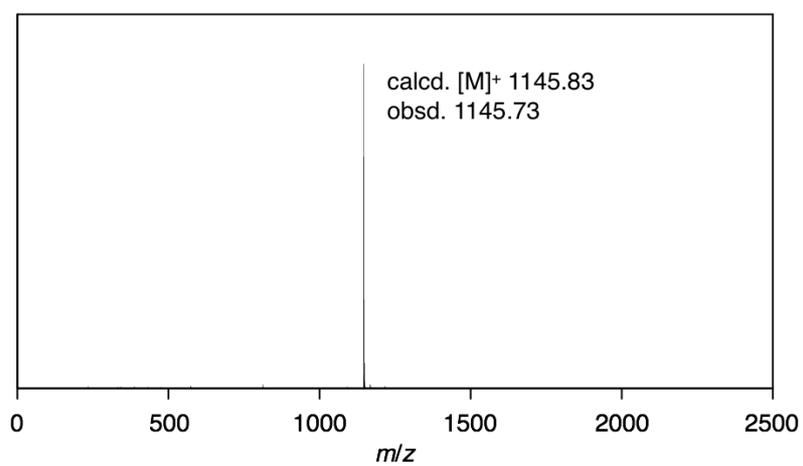


Figure S4. ESI-mass spectrum of $^{\text{CL}}\text{K}_3$.

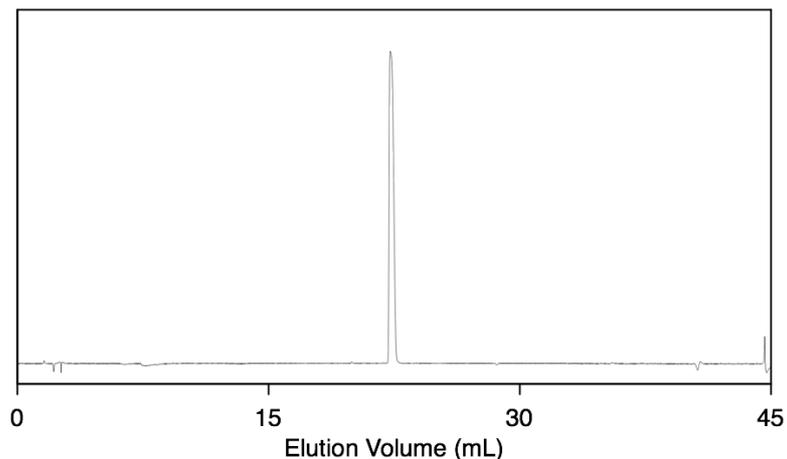


Figure S5. Analytical RP-HPLC trace of ^{CL}**K**₃. [^{CL}**K**₃] = 1.0 mM, Loading solvent; H₂O, eluent; H₂O–CH₃CN gradient containing 0.1% CF₃COOH (v/v), column; Phenomenex Jupiter 4U Proteo.

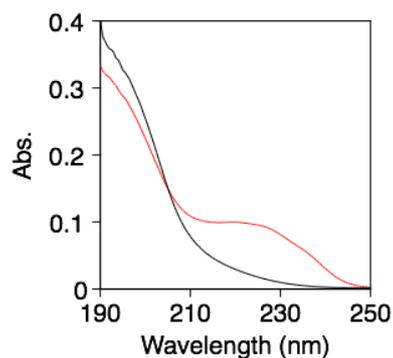


Figure S6. Electronic absorption spectra of ^{CL}**K**₃ (red, [^{CL}**K**₃] = 440 μM) and **K**₃ (black, [**K**₃] = 440 μM) in H₂O at 25 °C. An absorption at 225 nm is assignable to 1,3-diene moiety.

3. Methods

3.1. Covalent linking of $^{CL}K_3$

$^{CL}K_3$ was dissolved in filtered Milli-Q H₂O (8.8 mM), and the solution was sonicated for 20 min. To a 1 mL of $^{CL}K_3$ solution was added CH₃CN solution (10 μ L) of 2,2-dimethoxy-2-phenylacetophenone (DMPA, 51 mM) and the solution was incubated at 25 °C for 1 hour. The solution was transferred to a 1 cm quartz cuvette and ultraviolet light ($\lambda = 365$ nm, 8 W) was irradiated. During the photo-irradiation, temperature was maintained at 25 °C by use of an electric fan.

3.2. Preparation of cryogenic TEM samples

6.5 μ L of sample solution was deposited onto a Cu specimen grid covered with holey carbon support film (300 mesh, Electron Microscopy Science) and held in place with tweezers mounted on the Vitrobot. The specimen was blotted in an environment with 100% humidity at 22 °C (Blot time: 1 sec, wait time: 1 sec, drain time: 1 sec, blot force: 3 or 5, blot total: 3 or 5) and plunged into a liquid ethane reservoir cooled by liquid N₂. The vitrified samples were stored in liquid nitrogen and then transferred to a Gatan cryo-TEM holder.

3.3. Statistical analysis of fiber length

Contour length of nanofibers was determined by carefully tracing randomly selected 100 nanofibers by hand. Representative cryo-TEM images used for the analyses are shown in Figure S13–S16. L_n , L_w and σ (L_n : number-average length, L_w : weight-average length, and σ : standard deviation) were calculated based on following the equations reported previously (1–3) (L : length of nanofiber, N : number).^{10,11} L_w/L_n represents length dispersity.

$$L_n = \frac{\sum_{i=1}^n N_i L_i}{\sum_{i=1}^n N_i} \quad (1) \quad L_w = \frac{\sum_{i=1}^n N_i L_i^2}{\sum_{i=1}^n N_i L_i} \quad (2) \quad \frac{L_w}{L_n} - 1 = \left(\frac{\sigma}{L_n}\right)^2 \quad (3)$$

3.4. Preparation of vesicles for differential scanning calorimetry

A CHCl₃ (5 mL) solution of dipalmitoylphosphatidylcholine (DPPC) (7 mg, 10 μ mol) in a round-bottom flask was slowly evaporated to dryness at 25 °C, and the resulting film was further dried under high vacuum for longer than 2 hours. A lipid

film that developed on the interior surface of the round-bottom flask was allowed to be hydrated for 0.5 h at 60 °C with a HEPES buffer solution (1.0 mL, [HEPES] = 10 mM, [NaCl] = 100 mM, pH = 7.2), and the suspension was vortexed for 3 minutes. After 5 freeze-to-thaw cycles, the resultant dispersion was allowed to be extruded 20 times at 60 °C through a porous poly(vinylidene difluoride) membrane with a pore diameter of 100 nm, affording a dispersion of DPPC vesicles ([DPPC] = 10 mM).

3.5. Cell culture and Cytotoxicity assay

C2C12 mouse myoblasts were cultured in a humidified incubator (37 °C, 5% CO₂) with proliferation medium, consisting of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and 1% penicillin/streptomycin prior to reaching to 80% confluence.

To investigate the cytotoxicity of PA, cells were sub-cultured using 0.25% Trypsin-EDTA and seeded in 24 well plate (Denville Scientific Inc, Lot#20160020729A) at 25,000 cells/cm² with proliferation medium. After 24 hour incubation, medium was replaced with a fresh proliferation medium containing ⁶³K₃ PAs with different fiber length (n=3) PA solutions were prepared following the general sample preparation to a final concentration of 440 μM in water. The solutions of PAs were further diluted to 27.5 μM in proliferation medium. Cells grown in proliferation medium without PA were set up as control.

After 24 h culture, cell viability assay was performed using LIVE/DEAD[®] Viability/Cytotoxicity Kit according to manufacturer's instruction. In brief, cells were stained with calcein AM (2 μM) and Ethidium Homodimer-1 (4 μM) for 30 min at room temperature, and then rinsed with DPBS twice before fluorescent imaging performed on a cell imaging multi-mode microplate reader (Cytation 3, BioTek). Cell viability was quantified as a ratio of calcein positive cells over total number of cells.

As reported previously (Ref 22), the timescale and the conditions for biological assays do not change the length of PA fibers dramatically. We expect the difference in fiber length was maintained during the cell studies.

4. Analytical Data

4.1. Nile red assay

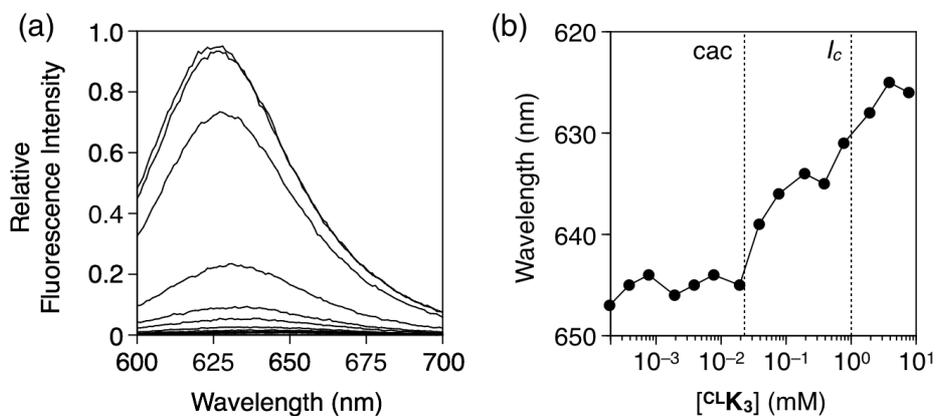


Figure S7. (a) Fluorescence spectrum for Nile red (250 nM) in H_2O at 25 °C with various concentrations of $^{CL}K_3$ ($\lambda_{ex} = 550$ nm). (b) Plots of Nile red fluorescence peak top as a function of $^{CL}K_3$.

4.2. FT-IR Spectroscopy

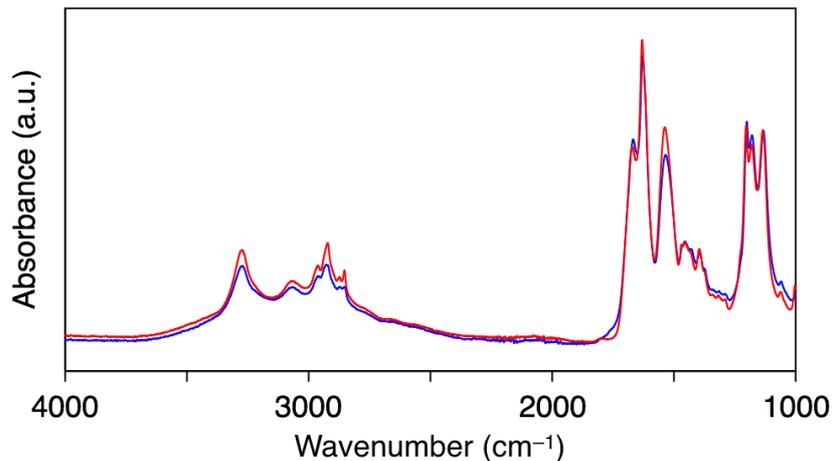


Figure S8. FT-IR spectra (4000–1000 cm^{-1}) of non-irradiated (red) and photo-irradiated (blue) $^{CL}K_3$ at 25 °C.

4.3. ^1H NMR Spectroscopy

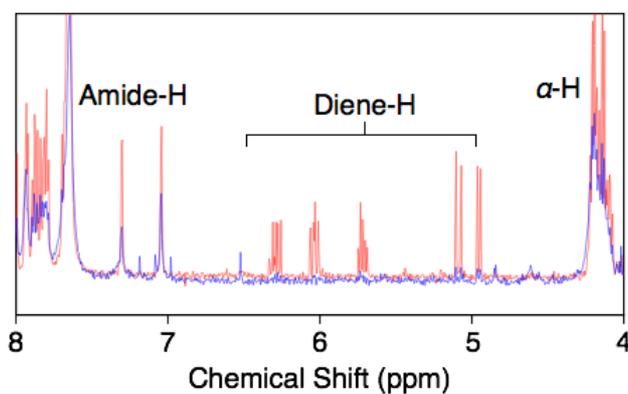


Figure S9. ^1H NMR spectra of non-irradiated (red) and photo-irradiated (blue) $^{\text{CL}}\text{K}_3$ in $\text{DMSO-}d_6$ at $25\text{ }^\circ\text{C}$.

4.4. Size-exclusion chromatography of photo-crosslinked $^{\text{CL}}\text{K}_3$

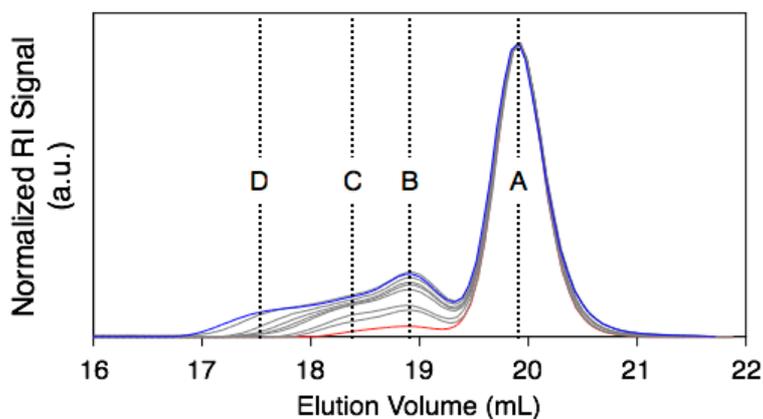


Figure S10. SEC-RI traces of $^{\text{CL}}\text{K}_3$ with HFIP as an eluent. RI signal intensity was normalized by $^{\text{CL}}\text{K}_3$ monomer peak (red trace: 0 h irradiation, blue trace: 144 h irradiation). Molecular weight for peaks A–D are calculated based on PMMA standard samples. Peak A: $M = 2677\text{ g mol}^{-1}$, Peak B: $M = 6090\text{ g mol}^{-1}$, Peak C: $M = 9490\text{ g mol}^{-1}$, Peak D: $M = 18700\text{ g mol}^{-1}$.

4.5. MALDI-TOF mass spectrometry of photo-crosslinked ${}^{\text{CL}}\mathbf{K}_3$

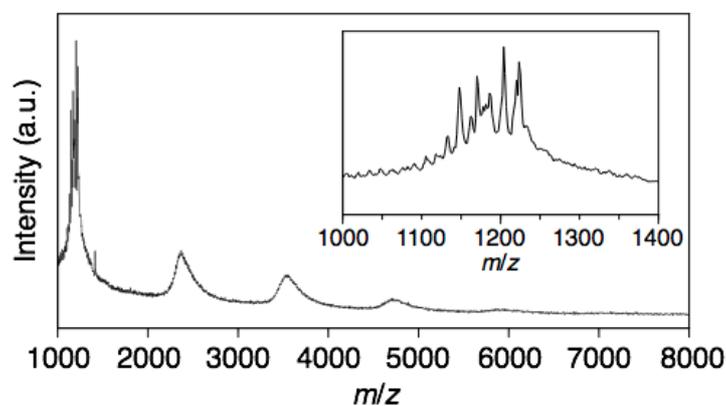


Figure S11. MALDI-TOF mass spectra of photo-irradiated ${}^{\text{CL}}\mathbf{K}_3$.

4.6. Cryogenic TEM images of nanofibers

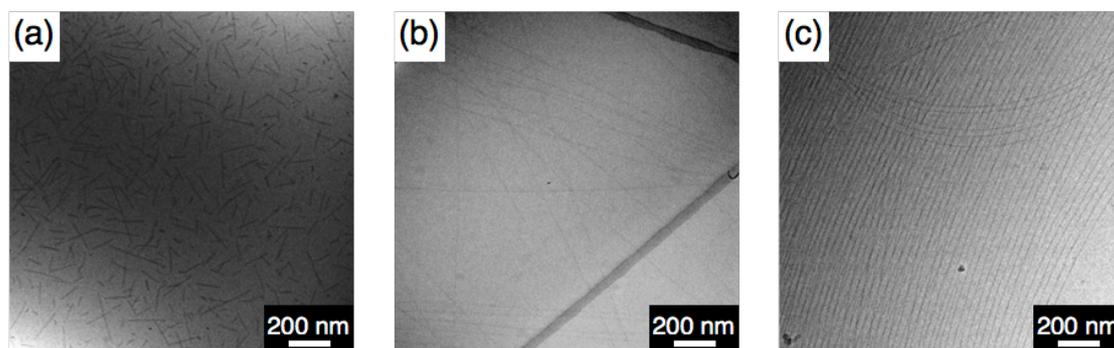


Figure S12. Cryogenic TEM images of non-irradiated ${}^{\text{CL}}\mathbf{K}_3$ under varieties of conditions. (a) Formation of short fibers below critical ionic strength. ${}^{\text{CL}}\mathbf{K}_3$ ($[{}^{\text{CL}}\mathbf{K}_3] = 0.44$ mM in H_2O) was annealed at 80 °C and then cooled to room temperature.²² (b and c) Formation of long fibers above critical ionic strength. (b) ${}^{\text{CL}}\mathbf{K}_3$ ($[{}^{\text{CL}}\mathbf{K}_3] = 0.44$ mM in aqueous NaCl (10 mM)) was annealed at 80 °C and then cooled to room temperature.²² (c) ${}^{\text{CL}}\mathbf{K}_3$ ($[{}^{\text{CL}}\mathbf{K}_3] = 4.4$ mM in H_2O) was annealed at 80 °C and then cooled to room temperature.²²

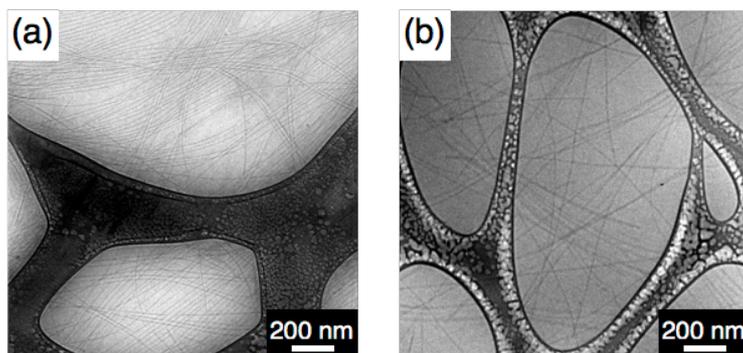


Figure S13. Cryogenic TEM images of ${}^{\text{CL}}\mathbf{K}_3$ ($[{}^{\text{CL}}\mathbf{K}_3] = 0.44$ mM) under varieties of conditions. (a) Non-irradiated infinitely long fibers and (b) photo-irradiated infinitely long fibers. Photo-irradiated and non-irradiated ${}^{\text{CL}}\mathbf{K}_3$ ($[{}^{\text{CL}}\mathbf{K}_3] = 4.4$ mM in H_2O) were annealed at 80 °C and then cooled to room temperature. Subsequent 10-fold dilution of the samples allowed to yield these infinitely long fibers.²²

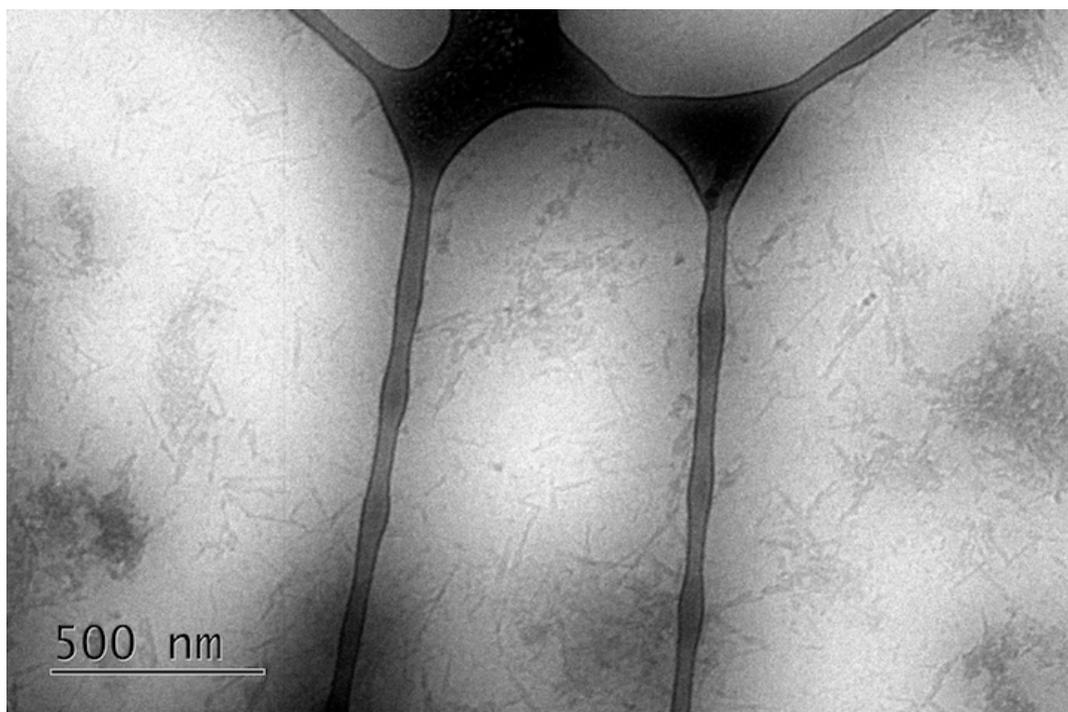


Figure S14. A cryogenic TEM image of ${}^{\text{CL}}\mathbf{K}_3$ ($[{}^{\text{CL}}\mathbf{K}_3] = 0.44$ mM in H_2O), without photo-irradiation.

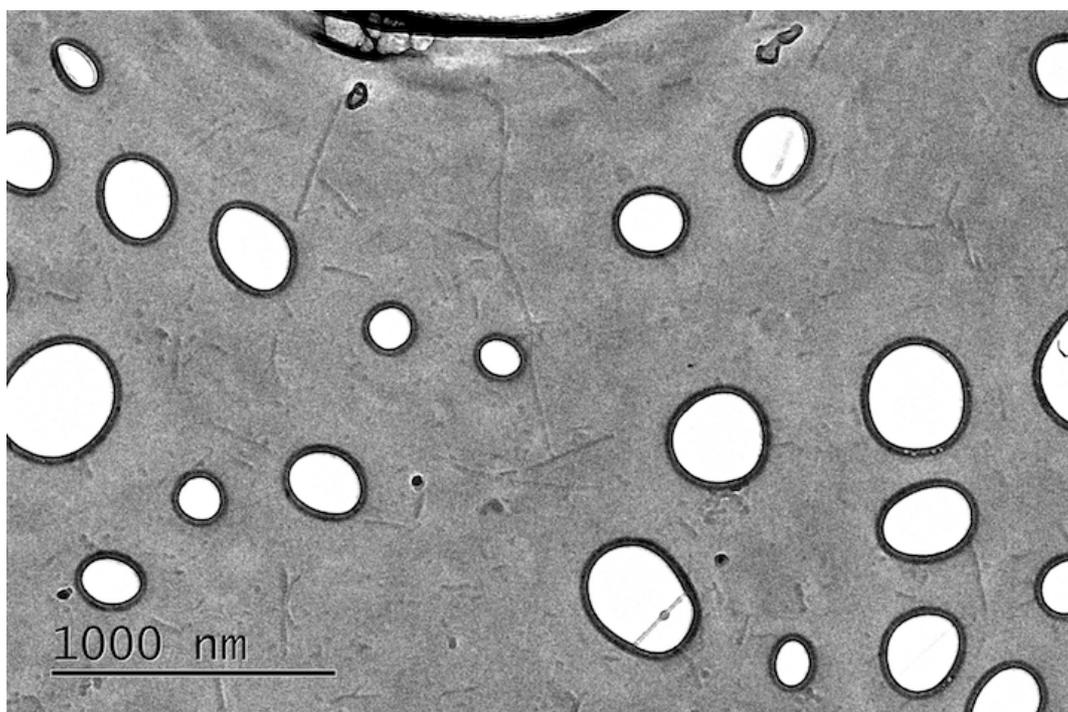


Figure S15. A cryogenic TEM image of ${}^{\text{CL}}\text{K}_3$ ($[{}^{\text{CL}}\text{K}_3] = 0.44 \text{ mM}$ in H_2O), photo-irradiated for 4 hours.

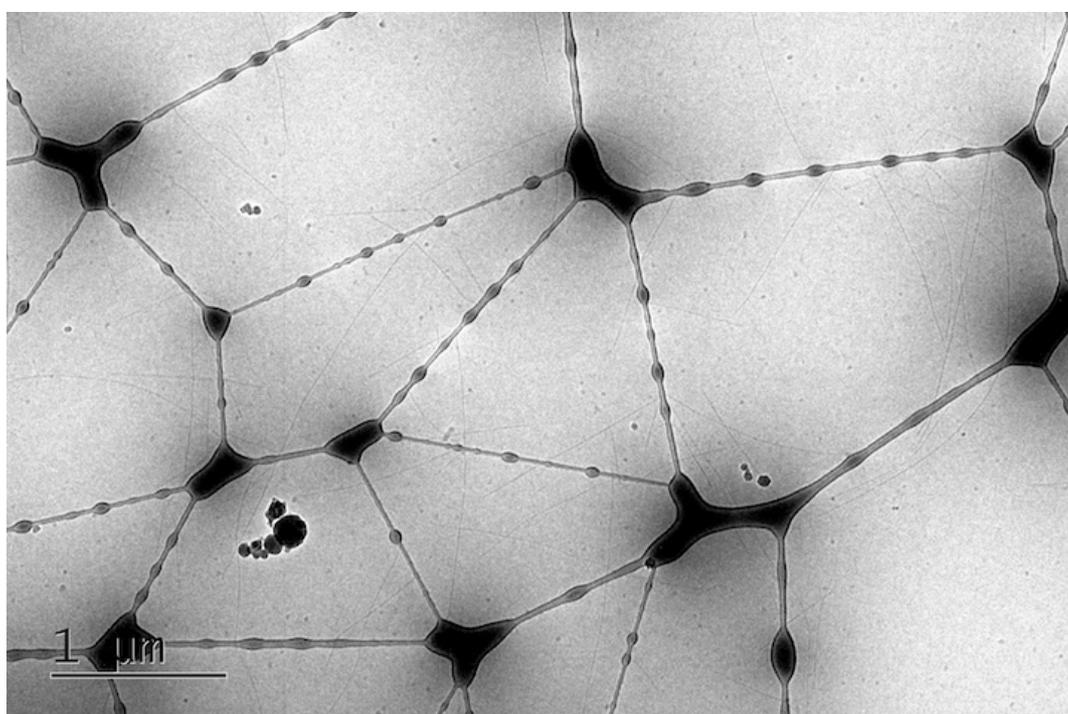


Figure S16. A cryogenic TEM image of ${}^{\text{CL}}\text{K}_3$ ($[{}^{\text{CL}}\text{K}_3] = 0.44 \text{ mM}$ in H_2O), photo-irradiated for 8 hours.

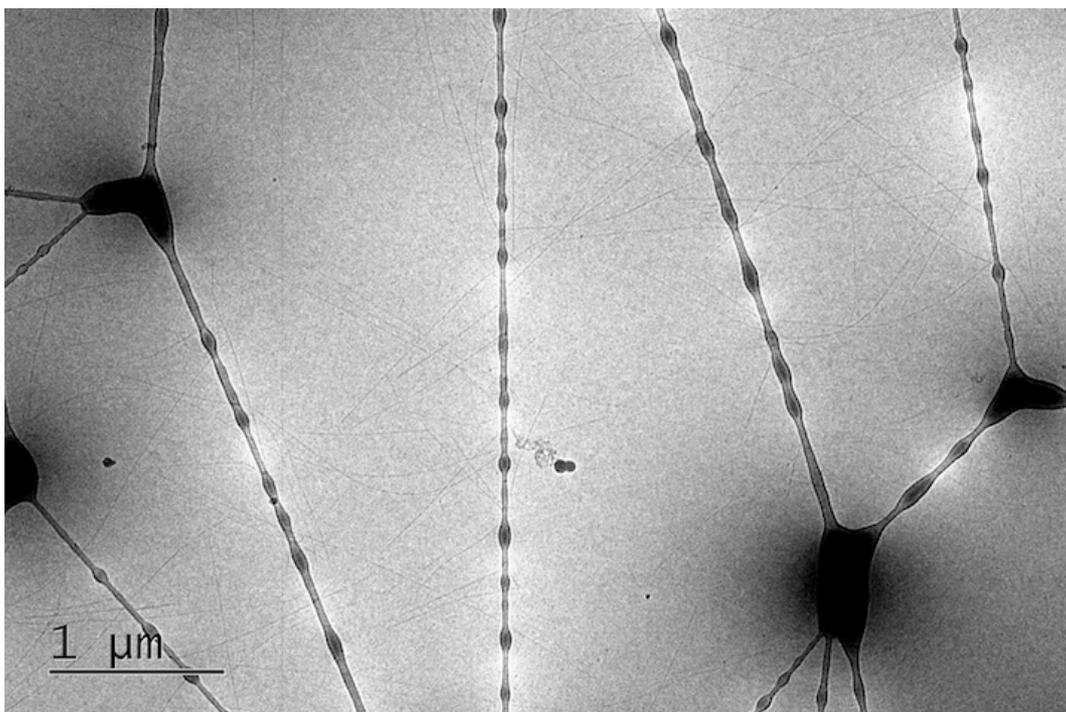


Figure S17. A cryogenic TEM image of ${}^{\text{CL}}\mathbf{K}_3$ ($[{}^{\text{CL}}\mathbf{K}_3] = 0.44 \text{ mM}$ in H_2O), photo-irradiated for 24 hours.

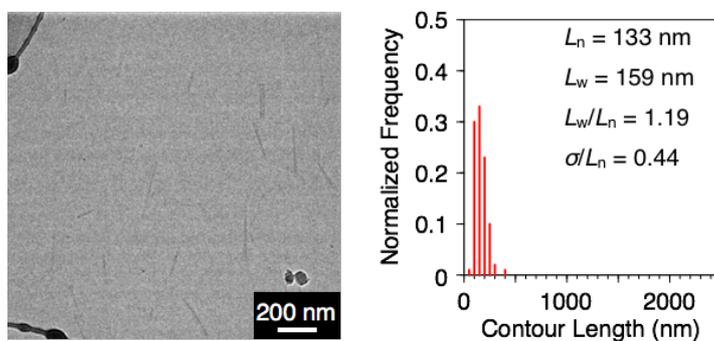


Figure S18. A cryogenic TEM image of \mathbf{K}_3 ($[\mathbf{K}_3] = 0.44 \text{ mM}$ in H_2O) and its corresponding histograms of the contour length of randomly selected 100 fibers. The sample solution was annealed at $80 \text{ }^\circ\text{C}$ and then cooled to room temperature.²²

4.7. Differential scanning calorimetries of liposomes

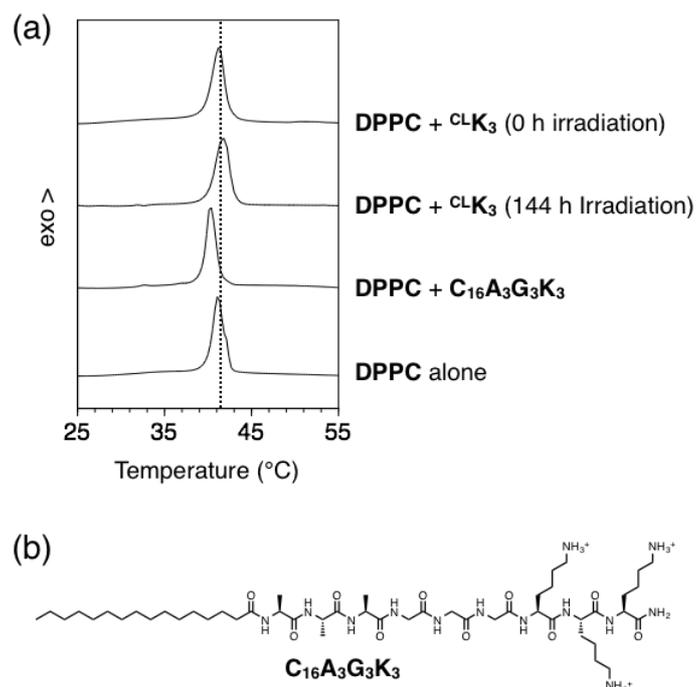


Figure S19. (a) Differential scanning calorimetry (DSC) traces on second heating of DPPC liposome-PA mixtures and DPPC liposome alone at a scan rate of $1\text{ }^{\circ}\text{C min}^{-1}$ ([DPPC] = 1.0 mM, [PA] = $170\text{ }\mu\text{M}$ in 10 mM HEPES 100 mM NaCl buffer solution). The phase transition temperature did not show changes in the presence of non-irradiated or irradiated ${}^{\text{CL}}\mathbf{K}_3$, indicating that no significant amount of ${}^{\text{CL}}\mathbf{K}_3$ monomers had incorporated into the membrane. $\mathbf{C}_{16}\mathbf{A}_3\mathbf{G}_3\mathbf{K}_3$, a previously reported membrane disrupting PA, was used as controls.³⁸ The mixture of DPPC liposomes and $\mathbf{C}_{16}\mathbf{A}_3\mathbf{G}_3\mathbf{K}_3$ showed lowering of transition temperature, indicating that $\mathbf{C}_{16}\mathbf{A}_3\mathbf{G}_3\mathbf{K}_3$ monomers had incorporated into the membrane, destabilizing the lipid bilayer structure. (b) Chemical structure of $\mathbf{C}_{16}\mathbf{A}_3\mathbf{G}_3\mathbf{K}_3$.

5. References

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