Supplementary Material (ESI) for Soft Matter

Self-Assembling Quniquethiophene-Oligopeptide Hydrogelators

by David A. Stone,[a] Lorraine Hsu[a] and Samuel I. Stupp*[abcd]

[a] Department of Chemistry, Northwestern University, 2145 Sheridan Rd., Evanston, Illinois 60208-3108, USA. Fax: +1 847 491 3010; Tel: +1 847 491 3002; E-mail: s-stupp@northwestern.edu
[b] Department of Materials Science and Engineering, Northwestern University, Evanston, Illinois, USA
[c] Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA
[d] Institute for BioNanotechnology in Medicine, Northwestern University, Chicago, Illinois, USA

1. Materials

Unless otherwise noted, all starting materials were obtained from commercial sources (Sigma-Aldrich, TCI, Alfa Aesar) and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded in a Varian Inova 500 (500 MHz for ^{1}H and 120 MHz for ^{13}C NMR) spectrometer using the solvent proton signal as standard. ESI mass spectrometry was performed on a LCQ Advantage. Preparative HPLC was performed on a Varian System using a Phenomenex Jupiter preparative column (10 μm size particle, 250 x 30.0 mm). Compound purity was analyzed by an analytical reverse-phase high-performance liquid chromatography (RP−HPLC) on an Agilent HP 1050 system equipped with a Phenomenex Jupiter analytical column (10 μm particle size, 150 × 4.6 mm). Conformation of mass and purity includes mass spectrometry and RP-HPLC. Synthesis of 5T-mDCA (scheme S1) was reported elsewhere first.[1]

1.1 5T-mDCA:

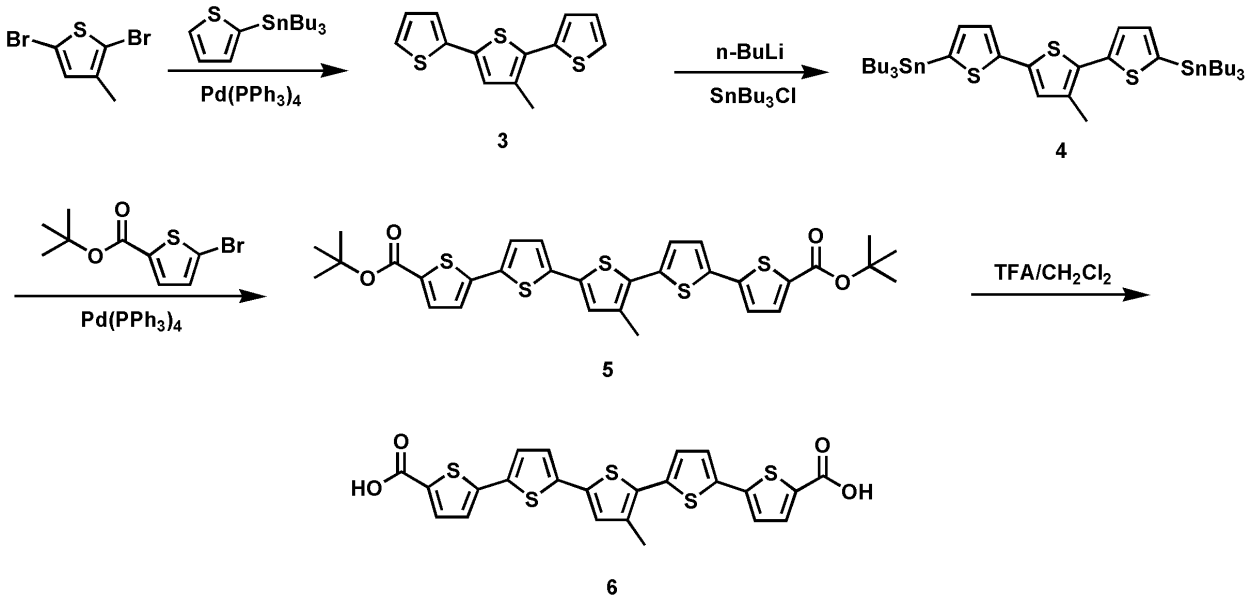


Figure S1. Synthetic scheme of 5T-mDCA

3-methyl-2,5-di(thiophen-2-yl)thiophene (3): In a dry and degassed flask, a solution of 2,5-dibromo-3-methylthiophene (1.0g, 4.0 mmol) in DMF (40 mL) was added 2-(tributylstannyl)thiophene (3.6 g, 9.6 mmol). The solution was sparged with N_2 gas for 20 minutes. Tetrakistriphenylphosphine palladium(0) (52 mg) was then added to the solution. After stirring at 100 °C for 16 h, the reaction was cooled to room temperature. The reaction mix was diluted with chloroform and extracted three times with 5% citric acid (aq). The organic layer was dried over Na_2SO_4 and the solvent was removed in vacuo. The crude product was purified by column chromatography (silica gel; chloroform/hexanes, 10:90) to yield 0.86 g (88%) of the compound as a yellow oil. ^{1}H NMR ($CDCl_3$, ppm): δ 7.32 (d, 1H, J = 5 Hz), 7.23 (d, 1H, J = 5 Hz), 7.19-7.18 (m, 2H), 7.10 (at, 1H, J = 4 Hz), 7.04 (at, 1H, J = 4), 7.00 (s, 1H), 2.40 (s, 3H). ^{13}C NMR ($CDCl_3$, ppm): δ 137.39, 136.59, 134.90, 134.79, 130.29, 128.15, 128.14,

127.76, 125.65, 125.38, 124.63, 123. 84, 15.79. ESI-MS (m/z): $[M]^+$ calcd for $C_{13}H_{10}S_3$, 262.41; found, 262.63.

2,5-bis(5-(tributylstannyl)thiophen-2-yl)-3-methylthiophene (4): A solution of **1** (0.30 g, 1.1 mmol) in anhydrous THF (30 mL) was cooled to -78 °C. *n*-Butyllithium in a 2.5M solution of cyclohexane (1.2 mL) was added and the reaction was stirred for 1 h while warming to room temperature. Tributyltin chloride (0.94 g, 2.9 mmol) was added and the reaction stirred for 16 h at room temperature. The reaction was diluted with hexanes and extracted three times with sat. $NaHCO_3$ (aq), dried with Na_2SO_4, concentrated and used without purification in the next step.

tert-butyl 5-bromothiophene-2-carboxylate: To a solution of 4-(*N,N*-dimethylamino)pyridine (0.86 g, 7.0 mmol), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (1.34, 7.0 mmol), tert-butyl alcohol (10 mL, 105 mmol) in dichloromethane (30 mL) was added 5-bromo-2-thiophenecarboxylic acid (1.0 g, 4.8 mmol). After stirring for 16 h at room temperature, the solvent was removed. The mixture was then dissolved in dichloromethane and extracted 3 times with 2M HCl (aq) followed by three times with sat. $NaHCO_3$. The organic layer was dried with Na_2SO_4 and concentrated to yield 1.16 g (92 %) of the desired product as a clear oil. ^{1}H NMR ($CDCl_3$, ppm): δ 7.47 (d, 1H, J = 4 Hz), 7.05 (d, 1H, J = 4 Hz), 1.57 (s, 9H). ^{13}C NMR ($CDCl_3$, ppm): δ 160.62, 137.12, 133.12, 130.92, 119.56, 82.55, 28. 41.

ditertbutyl ester 3-methyl-quinquethiophene (5): In a Nitrogen atmosphere, tert-butyl 5-bromothiophene-2-carboxylate (0.69 g, 2.6 mmol) was added to a solution of **2** (0.84 g, 1.1mmol) in DMF (40 mL). The solution was sparged with nitrogen gas for 20 min and

tetrakistriphenylphosphine palladium(0) (57mg) was added. The reaction stirred at 100 °C for 16 h. The reaction mixture was cooled to room temperature, diluted with chloroform, and extracted 3x with 5% citric acid (aq) to remove the DMF. After drying the organic layer over Na_2SO_4, the product was purified by column chromatography (silica gel; chloroform/hexanes, 65:35) to yield 0.62 g (90 %) of the desired product as a red-orange powder. ^{1}H NMR ($CDCl_3$, ppm): δ 7.64 (d, 1H, J = 4 Hz), 7.63 (d, 1H, J = 4 Hz), 7.24 (d, 1H, J = 4 Hz), 7.18 (d, 1H, J = 3.5 Hz), 7.18 (d, 1H, J = 3.5 Hz), 7.14 (d, 1H, J = 3.5 Hz), 7.12 (d, 1H, J = 4 Hz), 7.09 (ad, 2H, J = 4 Hz), 7.01 (s, 1H), 2.43 (s, 3H), 1.61 (s, 18H). ^{13}C NMR ($CDCl_3$, ppm): δ 161.56, 161.54, 143.22, 143.20, 137.41, 136.87, 136.31, 135.64, 135.56, 134.91, 134.00, 133.82, 133.80, 130.42, 128.68, 126.36, 125.98, 125.63, 124.74, 123.94, 123.88, 82.21, 82. 20, 25.50, 16.05. MALDI-TOF (m/z): $[M]^+$ calcd for $C_{31}H_{30}O_4S_5$, 626.89; found, 627.18.

dicarboxylic acid 3-methyl-quinquethiophene (5TmDCA) (6): In a solution 25 mL solution of Dichloromethane and TFA (5:1) **3** (50 mg, 0.79mmol) was stirred at room temperature for 2 h. The solvent was removed and the product was triturated three times with methanol and dried to yield 37 mg (92%) the desired product as a red solid. ^{1}H NMR (DMSO-d_6, ppm): δ 7.68 (d, 1H, J = 1.5), 7.67 (d, 1H, J = 1.5), 7.52 (d, 1H, J = 3.5), 7.48 (d, 1H, J = 4), 7.41 (d, 1H, J = 4), 7.39 (d, 1H, J = 4), 7.35 (d, 1H, J = 3.5), 7.31 (s, 1H), 7.28 (d, 1H, J = 4), 2.39 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 163.26, 146.78, 136.94, 136.54, 136.43, 135.69, 135.11, 135.03, 134.00, 133.47, 130.03, 129.91, 127.78, 127.61, 127.55, 127.45, 126.23, 125.56, 125.52, 16.18. ESI-MS (m/z): $[M]^+$ calcd for $C_{23}H_{14}O_4S_5$, 514.68; found, 515.53.

1.2 Peptidic Segment Synthesis:

All amino acid coupling in this chapter occurred under the same general conditions as follows. In a round bottom flask the free amine (1 equiv), free acid (1.2 equiv), HOBT (1.2 equiv), TEA (1.2 equiv) and EDC (1.2 equiv) were dissolved in dichloromethane. The reaction was stirred for 16 hours at room temperature. The reaction mixture was extracted three times with sat. $NaHCO_3$ (aq), followed by three times with 2M HCl. The organic layer was then dried with Na_2SO_4 and then removed by rotary evaporation. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH (95/5)) to yield the desired product.

All benzyl deprotection reactions followed the same procedure as follows. In a round bottom flask the carbobenzyloxy (Cbz) protected amine was dissolved in dichlormethane with 1 mL of methanol. To the solution, palladium on carbon (10 wt % Pd) was added (10 wt % cat loading). The reaction was allowed to stir for 16 hours under hydrogen. The reaction mixture was filtered through Celite to remove the catalyst, dried with Na_2SO_4 and then the solvent was removed by rotary evaporation. The crude product wass purified by column chromatography (silica gel, CH_2Cl_2/MeOH (95/5)) to yield the desired product. Conformation of product was based on ESI mass spectrometry because sever line broadening made ^1H NMR analysis difficult.

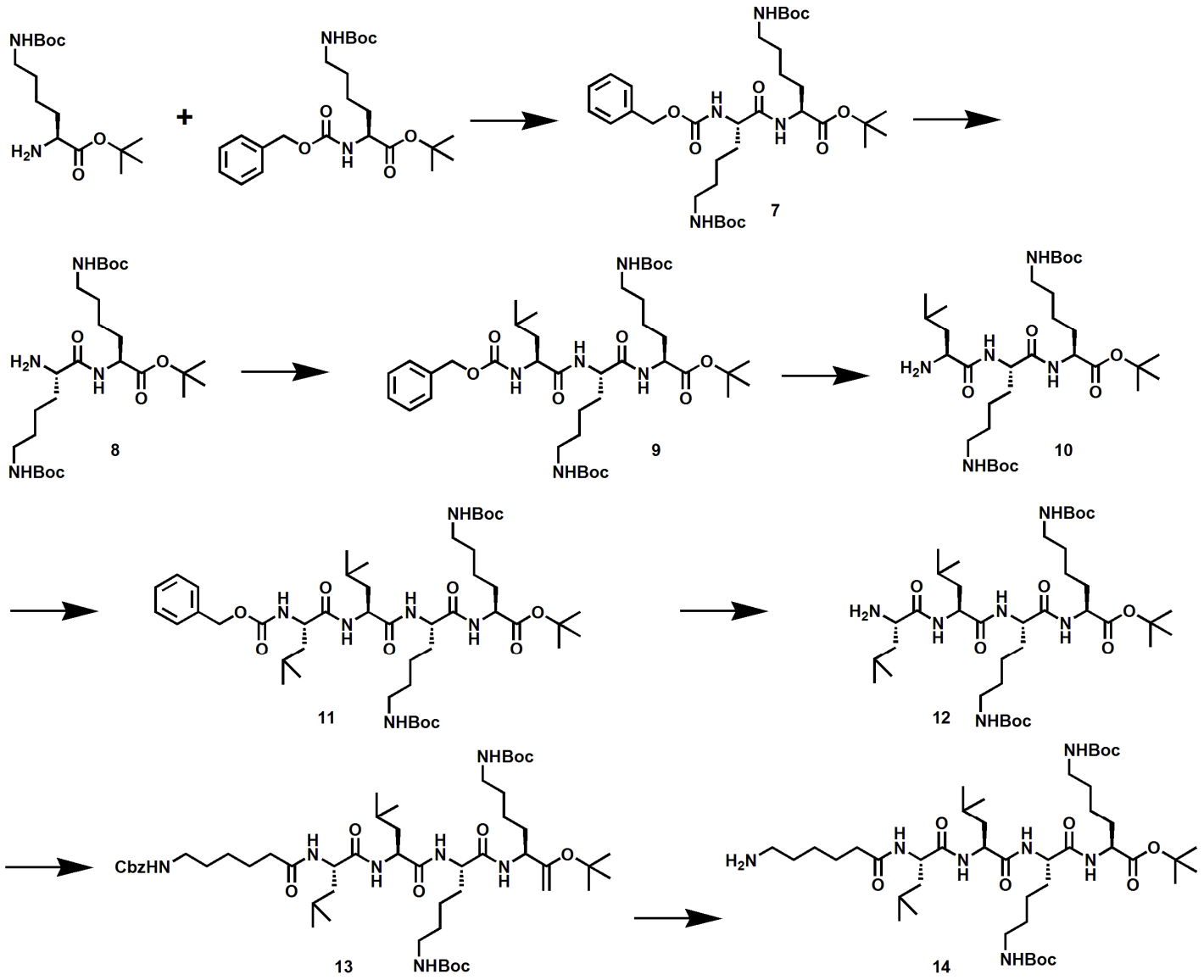


Figure S2. Synthetic scheme for peptidic segment.

Cbz-K(Boc)-K(Boc)-*t*Bu (7): Prepared by the general method described above using Cbz-K-(Boc)-OH (1.00 g, 3.0 mmol), H-K(Boc)-*t*Bu (0.95 g, 2.5 mmol), HOBT (0.40 g, 3.0 mmol), EDC (0.57 g, 3.0 mmol) and TEA (0.30 g, 3.0 mmol). Colum chromatography yielded 1.95 g (98 % yield) as a white solid. ESI-MS *m/z* calculated for $C_{34}H_{56}N_4O_9$, 664.83; found, 687.93 (M+Na).

Cbz-K(Boc)-K(Boc)-OH (8): Prepared by the general method above above for Cbz cleavage on Cbz-K(Boc)-K(Boc)-*t*Bu (1.95 g, 3 mmol). Colum chromatography yielded 1.56 g (quant.) as a white solid. ESI-MS *m/z* calculated for $C_{26}H_{50}N_4O_7$, 530.37; found, 531.80 (M+H).

Cbz-L-K(Boc)-K(Boc)-*t*Bu (9): Prepared by the general method described above using H-K-(Boc)-K(Boc)-*t*Bu (1.35 g, 2.5 mmol), Cbz-L-OH (0.79 g, 3.0 mmol), HOBT (0.38 g, 2.5 mmol), EDC (0.48 g, 2.5 mmol) and TEA (0.30 g, 3.0 mmol). Colum chromatography yielded 1.88 g (95 % yield) as a white solid. ESI-MS *m/z* calculated for $C_{40}H_{67}N_5O_{10}$, 777.49; found, 800.83 (M+Na).

H-L-K(Boc)-K(Boc)-*t*Bu (10): Prepared by the general method described above for Cbz deprotection on Cbz-L-K(Boc)-K(Boc)-*t*Bu (1.88 g, 2.5 mmol). Colum chromatography yielded 1.56 g (quant.) as a white solid. ESI-MS *m/z* calculated for $C_{32}H_{61}N_5O_8$, 643.45; found, 645.03 (M+2H).

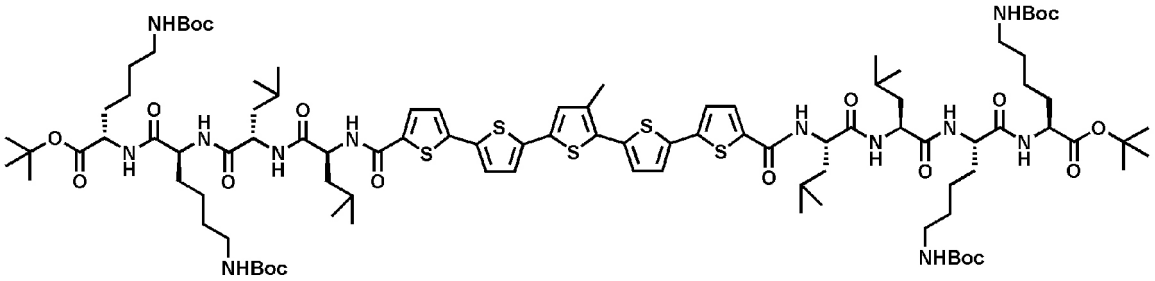
Cbz-L-L-K(Boc)-K(Boc)-*t*Bu (11): Prepared by the general method described above using H-L-K-(Boc)-K(Boc)-*t*Bu (0.56 g, 0.87 mmol), Cbz-L-OH (0.36 g, 1.0 mmol), HOBT (0.11 g, 0.87 mmol), EDC (0.17 g, 0.87 mmol) and TEA (0.08 g, 0.87 mmol). Colum chromatography yielded 0.77 g (99 % yield) as a white solid. ESI-MS *m/z* calculated for $C_{46}H_{78}N_6O_{11}$, 890.57; found, 914.17 (M+Na).

H-L-L-K(Boc)-K(Boc)-tBu (12): Prepared by the general method described above for Cbz deprotection on Cbz-L-L-K(Boc)-K(Boc)-*t*Bu (0.40 g, 0.45 mmol). Colum chromatography yielded 0.34 g (quant.) as a white solid. ESI-MS *m/z* calculated for $C_{38}H_{72}N_6O_9$, 756.54; found, 758.28 (M+2H).

Cbz-AHx-L-L-K(Boc)-K(Boc)-*t*Bu (13): Prepared by the general method described above using H-L-L-K-(Boc)-K(Boc)-*t*Bu (0.37 g, 0.45 mmol), Cbz-AHx-OH (0.16 g, 0.06 mmol), HOBT (0.06 g, 0.45 mmol), EDC (0.08 g, 0.45 mmol) and TEA (0.04 g, 0.45 mmol). Colum chromatography yielded 0.45 g (99 % yield) as a white solid. ESI-MS *m/z* calculated for $C_{52}H_{89}N_4O_{12}$, 1003.66; found, 1027.56 (M+Na+H).

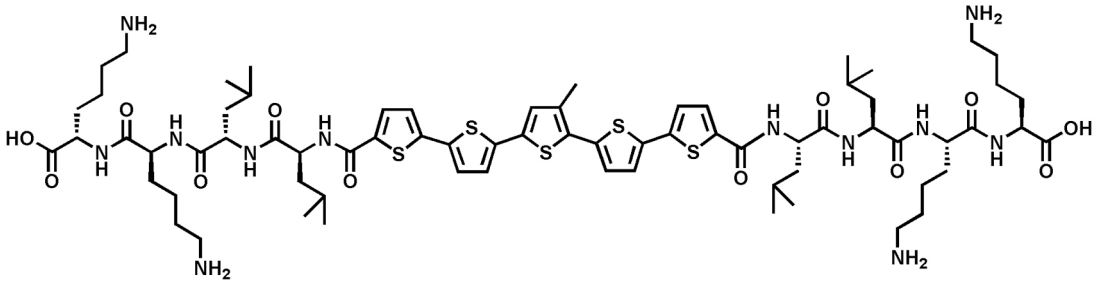
H-AHx-L-L-K(Boc)-K(Boc)-*t*Bu (14): Prepared by the general method described above for Cbz deprotection on Cbz-AHx-L-L-K(Boc)-K(Boc)-*t*Bu (0.45 g, 0.45 mmol). Colum chromatography yielded 0.39 g (quant.) as a white solid. ESI-MS *m/z* calculated for $C_{44}H_{83}N_7O_{10}$, 869.62; found, 871.55 (M+2H).

1.3 Peptidic Quinquethiophene Amphiphiles Synthesis:

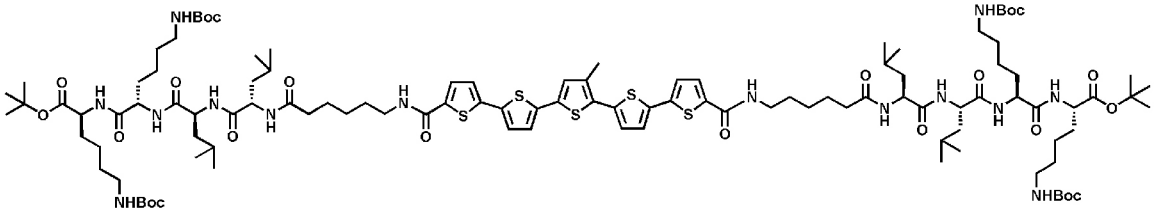


5TLLKK Protected: In a 100 mL round bottom flask 5TmDCA (0.11 g, 0.22 mmol), H-L-L-K(Boc)-K(Boc)-*t*Bu (0.40 g, 0.53 mmol), EDC (0.10 g, 0.53 mmol), HOBT (0.07 g, 0.53 mmol) and TEA (0.05, 0.53 mmol) were dissolved in DMF (50 mL). The reaction stirred for 16 hours at

room temperature. The DMF was removed by rotary evaporation and further dried under high vacuum. The crude product was purified by column chromatography (silica gel; chloroform/methanol, 95:05) to yield 0.31 g (73% yield) as a red-orange solid. The desired compound was not observed by ESI or MALDI-TOF, so the molecule was carried forward for deprotection.

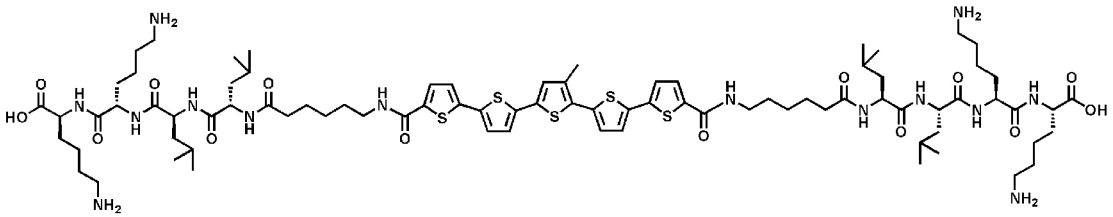


5TLLKK (2): The fully protected 5TLLKK derivative (0.10 g) was reacted with HBr (48% in water) in acetic acid in a 4:1 (25 mL) ratio for 10 min. The solution was then diluted with methanol (5 mL) and the product was precipitated with ether. The product was precipitated three times from methanol with either and collected by centrifugation. The product was purified by RP−HPLC. ESI-MS *m/z* calculated for $C_{71}H_{106}N_{12}O_{12}S_5$, 1478.67; found, 1479.33 $[M]^+$. HR-ESI-MS *m/z* calculated for $C_{71}H_{108}N_{12}O_{12}S_5^{2+}$, 740.34011; found, 740.34147.



5TAHxLLKK Protected: In a 100 mL round bottom flask 5TmDCA (0.10 g, 0.19 mmol), H-AHx-L-L-K(Boc)-K(Boc)-*t*Bu (0.39 g, 0.45 mmol), EDC (0.09 g, 0.45 mmol), HOBT (0.06 g, 0.45 mmol) and TEA (0.05, 0.45 mmol) were dissolved in DMF (50 mL). The reaction was

stirred for 16 h at room temperature. The DMF was removed by rotary evaporation and further dried under high vacuum. The crude product was purified by column chromatography (silica gel; chloroform/methanol, 95:05) to yield 0.28 g (67% yield) as a red-orange solid. The desired compound was not observed by ESI or MALDI-TOF, so the molecule was carried forward for deprotection.



5TAHxLLKK (1): The fully protected 5TAHxLLKK (0.10 g) derivative was reacted with HBr (48% in water) in acetic acid in a 4:1 (25 mL) ratio for 10 min. The solution was then diluted with methanol (5 mL) and the product precipitated with ether. The product was precipitated three times from methanol with either and collected by centrifugation. The product was purified by RP−HPLC. ESI-MS *m/z* calculated for $C_{83}H_{128}N_{14}O_{14}S_5$, 1706.86; found, 1706.30 $[M]^+$. ESI-MS *m/z* calculated for $C_{83}H_{130}N_{14}O_{14}S_5^{2+}$, 853.24218; found, 853.42346 $[M]^{2+}$.

2. Analysis

UV-Vis absorption spectra were collected on a Cary 500 spectrometer in double beam mode. All samples were prepared and measured at 200 μM in a 0.1 cm pathlength quartz cuvette. Fluorescence spectra were recorded on a PC1 Spectrofluorometer in right angle geometry using monochromators with a FWHM of 8 nm for both the excitation and emission. All spectra were collected by exciting at the absorption maxima. CD spectroscopy was performed on a model J-714 Jasco Circular Dichroism Spectrometer at the same concentration as UV-Vis spectroscopy.

For temperature dependent measurements, the samples were allowed to equilibrate for 5 min at the specified temperature before measurement.

All gels were formed by dissolving the derivative at the desired concentration. The samples were then heated to 80 °C and allowed to cool to room temperature. Gel formation was considered to occur when the solution was stable to vial inversion.

AFM measurements were taken on a Nanoscope Multimode ScanningProbe Microscope. Films were cast from a dilute aqueous solution (0.05 wt %) on to a freshly cleaved mica surface. Transmission electron microscopy (TEM) was performed on a Hitachi 8100 operating at 100 kV on holey carbon coated Cu TEM grids. All TEM samples were stained with uranyl acetate for 15 minutes.

3. Supplemental Figures

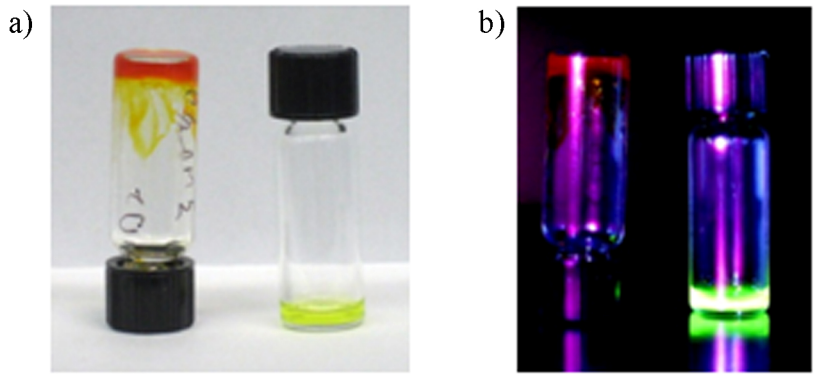


Figure S3. a) Photograph of 5TAHxLLKK as a 1 wt% gel in water (left) and a 1 wt% solution in methanol (right). b) Photograph of 5TAHxLLKK as a 1 wt% gel in water (left) and a 1 wt% solution in methanol (right) illuminated at 365 nm. A strong emission is observed in the methanol solution, but emission is quenched in the aqueous gel.

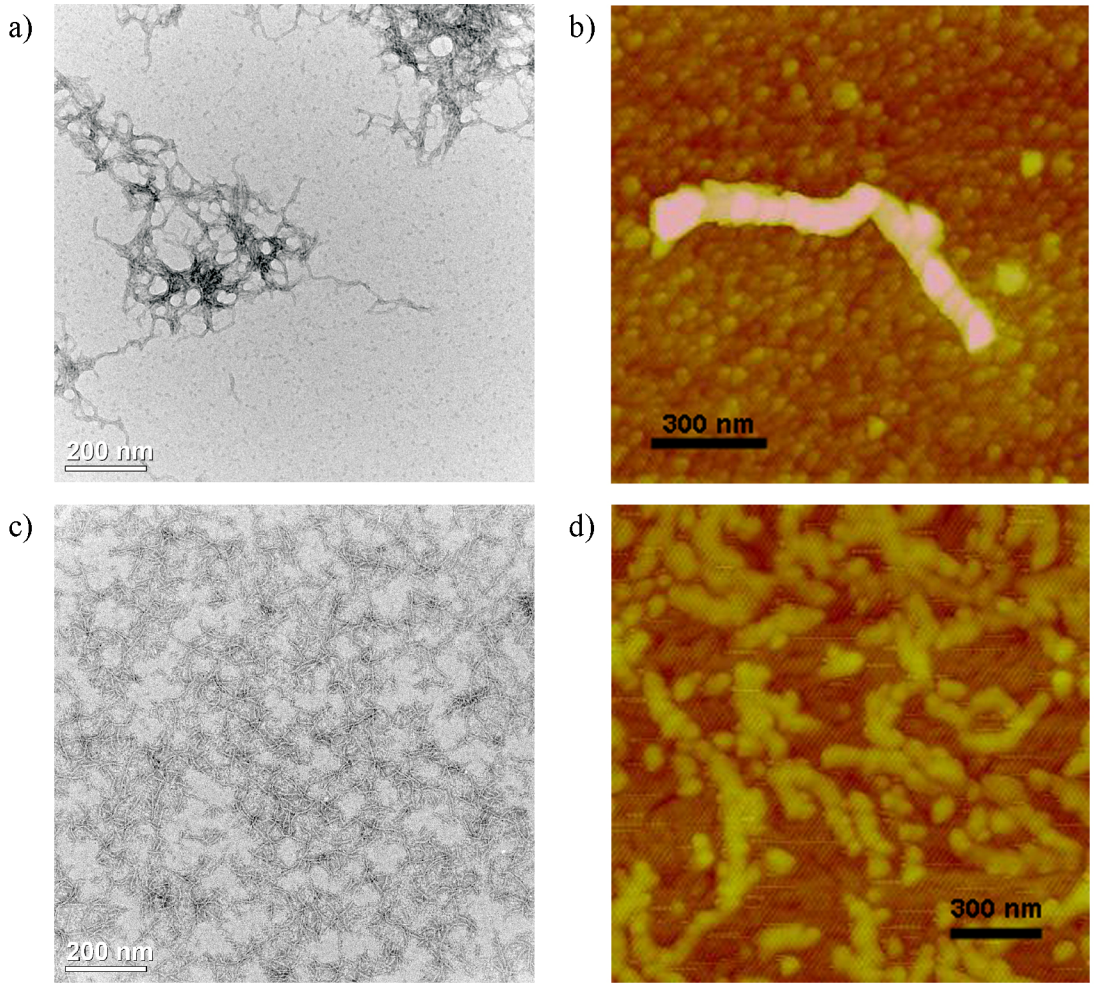


Figure S4: a) TEM microscopy of 5TAHxLLKK at lower magnification. b) AFM micrograph of a single nanostructure on mica. c) TEM micrograph of 5TLLKK at lower magnification. d) AFM micrograph of 5TLLMM at higher magnification.

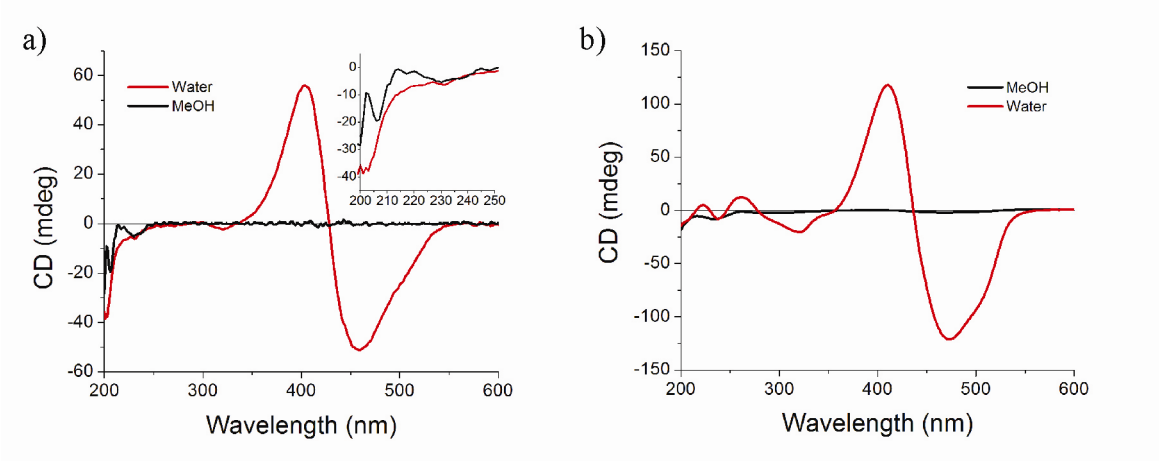


Figure S5. a) CD spectrum of 5TAHxLLKK (**1**) in water and methanol. b) CD spectrum of 5TLLKK (**2**) in water and methanol. Concentration for all spectra recorded is 200 μM.

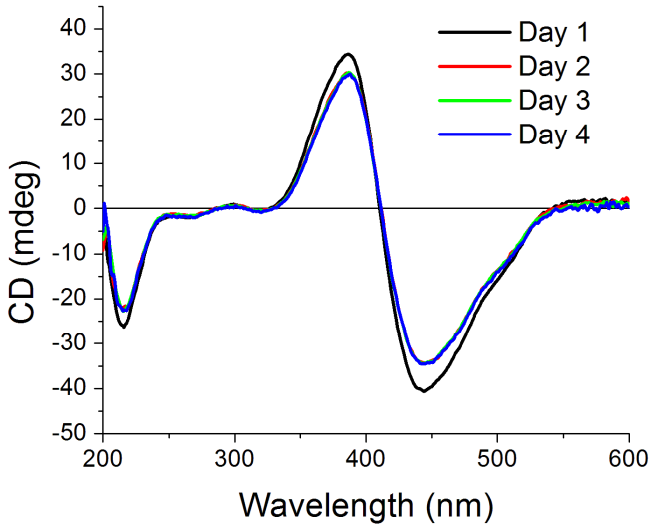


Figure S6. CD spectra of 5TAHxLLKK (**1**) in water measured after annealing to 80 °C and cooling to room temperature. Sample was kept at room temperature while aging after the initial annealing process.

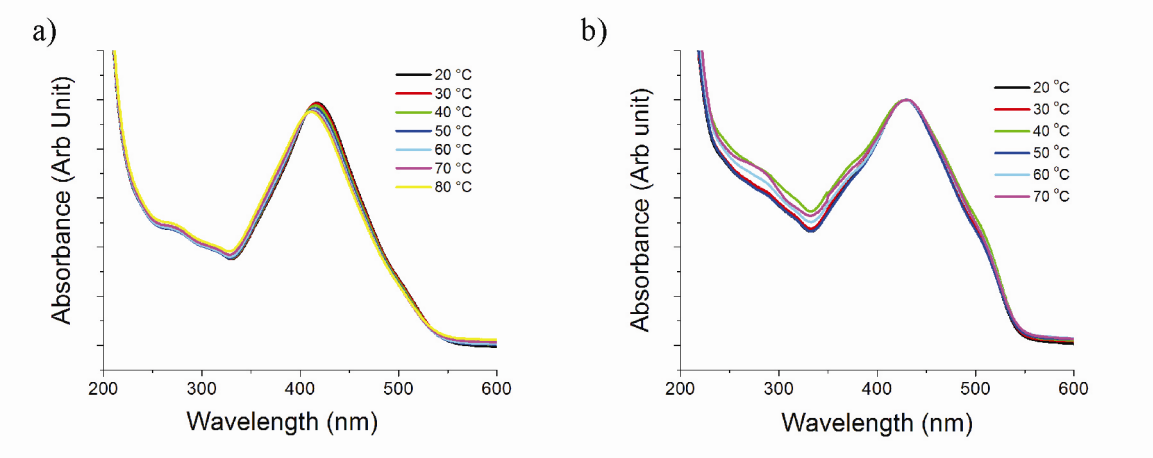


Figure S7. a) Variable temperature absorption of 5TAHxLLKK (**1**) in water. b) Variable temperature absorption of 5LLKK (**2**) in water. Sample concentration is 200 μM.

1. M. Sofos, J. Goldberger, D. A. Stone, J. E. Allen, Q. Ma, D. J. Herman, W. W. Tsai, L. J. Lauhon and S. I. Stupp, *Nature Materials*, 2009, **8**, 68-75.