Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Light-Driven Dissipative Self-Assembly of a Peptide Hydrogel

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Supporting Information

General Methods and Materials

4-Azinobenzoic acid, 3-methyl-2-butanone, 2-hydroxy-4-nitrobenzaldehyde, piperazine, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), trifluoroacetic acid (TFA), triethylsilane (TES) and deuterated solvents were purchased from Oakwood Chemical. Methyl iodide (MeI), N,N-diisopropylethylamine (DIPEA), solvents and acids were purchased from Fisher Scientific. The Fmoc-amino acids, and 1-hydroxybenzotriazole (HOBt), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) were purchased from Chem-Impex Int'l Inc. 1,3-Dimethoxybenzene (DMB) and acetic anhydride was purchased from Sigma-Aldrich. Reverse-phase HPLC was performed on a ThermoScientific Ultimate 3000 HPLC using a Waters C8 XBridge 10 uM 9x10mm prep. column w/ Guard Cartridge or a ThermoScientific C18 Acclaim120 5uM 4.6x100mm analytical column. NMR spectra were obtained on Bruker AMX 400 spectrometers and masses on a Bruker microTOF ESI-MS.

Solid-Phase Peptide Synthesis (SPPS)

General Methods and Protocols. Peptide synthesis was performed on Rink amide solid-phase resin (~0.5 mmol/g loading) using fritted syringes with a Teflon stopcock and stoppered with a septum. A wrist-action shaker was used to agitate the syringes and rinses were performed via vacuum filtration. Dry resin was allowed to swell in CH_2Cl_2 by shaking for 1 h before proceeding with the next step. A Kaiser test was performed after each coupling to ensure completion of each coupling step. Fmoc-Deprotections of the Fmoc protection groups were achieved using 3 x 5 min treatment with 2% DBU (2%) / piperazine (5%) in DMF. After each deprotection and coupling step, the resin was rinsed with DMF (3x), MeOH (2x), and CH_2Cl_2 (2x). Deprotection of the Mtt protecting groups were achieved by 3-fold treatment with TFA (2%)/1% TES (1%) in CH_2Cl_2 for

5 min, followed by washing the resin with CH_2Cl_2 (3x). Simultaneous deprotection of the Boc groups and cleavage of the peptide from the resin was achieved by treatment with 5% DMB (5%)/5% TES (5%)/TFA (90%) for 2 h. The solution drained from the syringe and diluted with 100 mL of cold diethyl ether. Additional cold diethyl ether was added to induce precipitation of the crude peptide and then centrifuged (10 min, 5000 RPM) and the remaining supernatant removed by decanting. The crude peptide was dried by lyophilization then purified using prep-scale reverse-phase HPLC on C8 reverse-phase column using an CH_3CN/H_2O eluant, lyophilized, and stored at r.t. in darkness.

Fmoc-KK(SP)KF-NH_2 (1)

The Rink resin (0.2 mmol, 1 eq) was swollen in CH_2Cl_2, and the Fmoc was deprotected using the standard protocol. A solution of Fmoc-Phe-OH (0.16 g, 0.4 mmol, 2 eq) in 5:1 DMF:DCM was treated with HOBt (0.081 g, 0.6 mmol, 3 eq) and HBTU (0.23 g, 0.6 mmol, 3 eq) for 5 min., followed by DIPEA (0.14 mL, 0.8 mmol, 4 eq) for 1 min, which produced in a light yellow solution. The solution was then added to the resin, stoppered, and shaken for 14 h. Afterwards, the resin was washed, Fmoc-deprotected, and rinsed following the standard protocol. A solution of Fmoc-Lys(Boc)-OH (0.28 g, 0.6 mmol, 3 eq) in 5:1 DMF:CH_2Cl_2 was prepared in a separate vial, then HOBt (0.081 g, 0.6 mmol, 3 eq) and HBTU (0.23 g, 0.6 mmol, 3 eq) were added simultaneously. After 5 min., DIPEA (0.14 mL, 0.8 mmol, 4 eq) was added, resulting in a pale yellow solution. The solution was then added to the resin, stoppered, and shaken for 14 h. Afterwards, the resin was washed, Fmoc-deprotected, then rinsed again following the standard protocol. A solution of Fmoc-Lys(Mtt)-OH (0.38 g, 0.6 mmol, 3 eq) in 5:1 DMF:CH_2Cl_2 was prepared in a separate vial, then HOBt (0.081 g, 0.6 mmol, 3 eq) and HBTU (0.23 g, 0.6 mmol, 3

eq) were added simultaneously. After 5 min., DIPEA (0.14 mL, 0.8 mmol, 4 eq) was added, thoroughly mixed and let set for 1 min, resulting in a light yellow color solution. The solution was then added to the resin, stoppered, and shaken for 14 h. Afterwards, the resin was washed, Fmoc-deprotected, then rinsed again. The Fmoc-Lys(Boc)-OH coupling was repeated using the same following the same procedure. After rinsing the resin and deprotecting the Mtt group, using the standard protocol, a solution of 1',3',3'-trimethyl-5'-carboxy-6-nitrospiro[2H-benzopyran-2,2'-3H-indole] (**SP-CO_2H)**[1] (0.22 g, 0.6 mmol, 3 eq)[1] in 5:1 DMF:CH_2Cl_2 was prepared in a separate vial and treated with HOBt (0.081 g, 0.6 mmol, 3 eq) and HBTU (0.23 g, 0.6 mmol, 3 eq) for 5 min, followed by DIPEA (0.14 mL, 0.8 mmol, 4 eq), resulting in purple-red solution. After 1 min., the solution was then added to the resin, stoppered, and shaken for 14 h. After rinsing, the Boc-protecting groups were removed, and the peptide was cleaved simultaneously from the resin. The crude peptide was precipitated in cold diethyl ether, centrifuged and purified to yield **1**. Prep reverse-phase HPLC on a C8 reverse-phase column with CH_3CN/H_2O with 0.1% TFA as eluant using the following gradient: 10 mL/min; 1 min. at 25% CH_3CN:; 1-20 min up to 70% CH_3CN; 20-22 min up to 100% CH_3CN; 22-25 min at 100% CH_3CN; 25-30 min down to 25% CH_3CN. Two peaks were isolated (13 min, **1**-MCH^+; 18 min, **1**-SP). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.13 (d, J = 2.8 Hz, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.82 (d, J = 7.5 Hz, 2H), 7.74 (dd, J = 8.2, 1.8 Hz, 1H), 7.69-7.62 (m, 3H), 7.41 (t, J = 7.4 Hz, 2H), 7.33 (t, J = 7.5 Hz, 2H), 7.29-7.25 (m, 4H), 7.24-7.17 (m, 2H), 7.12 (d, J = 10.4 Hz, 1H), 6.77-6.69 (m, 1H), 6.60 (d, J = 8.2 Hz, 1H), 5.96 (d, J = 10.4 Hz, 1H), 4.62 (dd, J = 8.8, 5.4 Hz, 1H), 4.39-4.17 (m, 6H), 4.13 (dd, J = 8.7, 6.1 Hz, 1H), 3.49-3.36 (m, 2H), 3.19 (dd, J = 13.9, 5.3 Hz, 1H), 3.01-2.84 (m, 6H), 2.79 (s, 3H), 1.91-1.56 (m, 15H), 1.55-1.41 (m, 5H), 1.40-1.28 (m, 5H), 1.18 (s, 3H) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 176.09, 175.50, 174.44, 171.30, 161.49, 159.47, 152.96, 146.11, 145.95, 143.47, 139.25, 138.55,

131.25, 130.30, 129.73, 128.65, 127.61, 127.03, 123.02, 122.88, 121.87, 121.18, 117.24, 108.56, 108.19, 68.92, 57.46, 56.48, 56.07, 55.59, 53.99, 41.33, 39.75, 33.20, 33.06, 32.95, 31.02, 29.83, 28.95, 28.95, 28.75, 27.03, 25.18,24.62, 24.40, 21.04 ppm. ESI-MS Calculated for $C_{62}H_{75}N_{10}O_{10}$ [M+H]+ = 1119.5668, found = 1119.5662.

Supporting Information

Methods

Spectroscopy and Rate Constants

Measurement of Thermal 1-SP1-MCH$^+$ Rate Constants

NMR spectra were obtained on Bruker AMX 400 spectrometers and masses on a Bruker microTOF ESI-MS. UV-Vis spectroscopy studies were conducted on a Shimadzu UV-2450 Spectrometer with a TCC-240A Temperature-Controlled Cell Holder using a 1 mm path length quartz cuvette. First order rate constants for the thermal conversion of **1**-SP to **1**-MCH$^+$ were determined by first converting measured MCH absorbance intensity at 415 nm of MCH$^+$ to concentration via a standard curve, then subtracting from the initial concentration [SP_0] to find the time dependent [SP] concentration. The kinetic traces were fitted to a monoexponential rate expression according to eq. 4, converted from the standard first order rate equation eq. 1.

$$A = A_0\, e^{-kt} \quad (1)$$

$$[\mathrm{SP}] = [\mathrm{SP}]_0\, e^{-kt} \quad (2)$$

$$[\mathrm{SP}]_0 - [\mathrm{MCH}^+] = [\mathrm{SP}]_0\, e^{-kt} \quad (3)$$

$$[\mathrm{MCH}^+] = [\mathrm{SP}]_0 - [\mathrm{SP}]_0\, e^{-kt} \quad (4)$$

$$\ln \frac{[\mathrm{SP}]_0 - [\mathrm{MCH}^+]}{[\mathrm{SP}]_0} = -kt \quad (5)$$

A plot of eq. 5 gave a line with a linear portion possessing a slope equal to the desired rate constant. CD spectra were recorded on a Jasco CD spectrometer under a nitrogen atmosphere using a 1 mm path length quartz cuvette. Fluorescence spectra were recorded on a Schimadzu RF-5301 PC Spectrofluorometer using a 200 uL 3 mm path length quartz cuvette.

Supporting Information

ThT Binding Experiments

A 2.5 mM ThT stock solution was prepared by adding 8 mg ThT to 10 mL PBS and filtered through 0.2 μm syringe filter and then kept in dark in 4°C fridge. The stock solution was then diluted to 0.05 mM with PBS solution immediately before the analysis to make the working solution. The fluorescence intensity of a 200 uL working solution was measured with excitation at 440 nm (slit width 5 nm) and emission at ~490 nm (slit width 10 nm). Then 5 μL of the hydrogel (formed at 10 mM (90 mmTFA) over 7 days) was dispersed in the working solution and mixed thoroughly for 3 min. The corresponding fluorescence was measured and recorded. Another 5 μL of hydrogel was added subsequently, mixed thoroughly for 3 min and the fluorescence emission spectrum was measured. An increase in fluorescence response indicated amyloid-type nanofibers.

Microscopy

Transmission Electron Microscopy (TEM) images were obtained using FEI Tecnai G2 Biotwin TEM- negative staining. Samples were prepared by placing a drop of sample on a clean parafilm surface, floating a copper 200 mesh TEM grid (Ted Pella, Inc.) on top for 2-3 min, then removing the excess liquid with a clean Kimwipe. Staining with 2% aqueous uranyl acetate (filtered through a 0.2 ųm syringe filter) was performed by placing a drop of stain solution on a clean parafilm surface then floating the sampled copped grid on top for 40-50 seconds then removing the excess liquid with a clean Kimwipe.

Atomic Force Microscopy (AFM) images were collected on a NanoScope IIIa device at ambient temperature. 0.3 mM diluted sample solutions (diluted from 3 mM stock assembled solns.) were dropped on freshly cleaved mica and allowed to dry for 10 min, then washed with water- the excess

dabbed off with the side of a Kimwipe. Samples were allowed to fully dry. The AFM tip was a Model: SCANASYST-AIR from Bruker with a silicon tip on nitride lever; cantilever T- 600nm. The scanning speed was at a line frequency of 1.0 Hz, and the original images were sampled at a resolution of 512 × 512 pixels. Nanoscope software was used to analyze images.

Dynamic Self-Assembly

For room temperature preparations in the light, a desk lamp outfitted with a broad-spectrum visible LED lightbulb (60W equivalent, 9.5W actual; 800 lumens) was focused at the vials containing solutions of **1**-SP/MCH^+ solutions. For room temperature preparations in the dark, samples were typically pre-heated at 60°C/darkness until isomerized to **1**-MCH^+, then removed from the heat source, wrapped in foil, and placed inside a drawer or other suitably dark area. For temperatures above room temperature, a lightbox was prepared by connecting two polypropylene bins together coating the inside with aluminum foil, then taping a 16.4 ft strand of 300 broad-spectrum visible LED lights (72W, 3600 lumens) along the sides and top of the box (Fig. S1). Lastly, a front cover of foil-lined cardboard was affixed to prevent light from escaping. The box was large enough to hold a small hotplate and small oil bath which was filled with colorless and very clear silicone oil. The LED strip was connected to an external switch for ease of turning on or off.

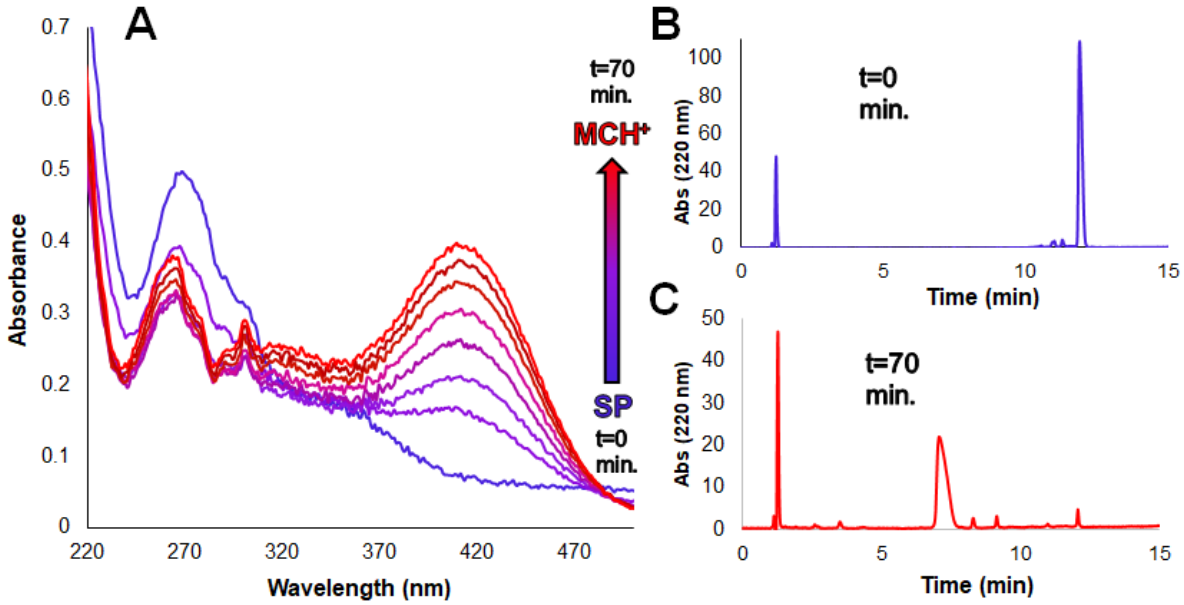


Figure S1. A) UV-Vis spectra of **1**-SP (0.3 mM, 90 mM TFA in water) opening at 60°C/darkness over 70 min showing conversion from **1**-SP to **1**-MCH^+, with corresponding reverse-phase analytical HPLC traces of the sample (C8 reverse-phase column using a CH_3CN/H_2O gradient) at B) 0 min and **C)** 70 min.

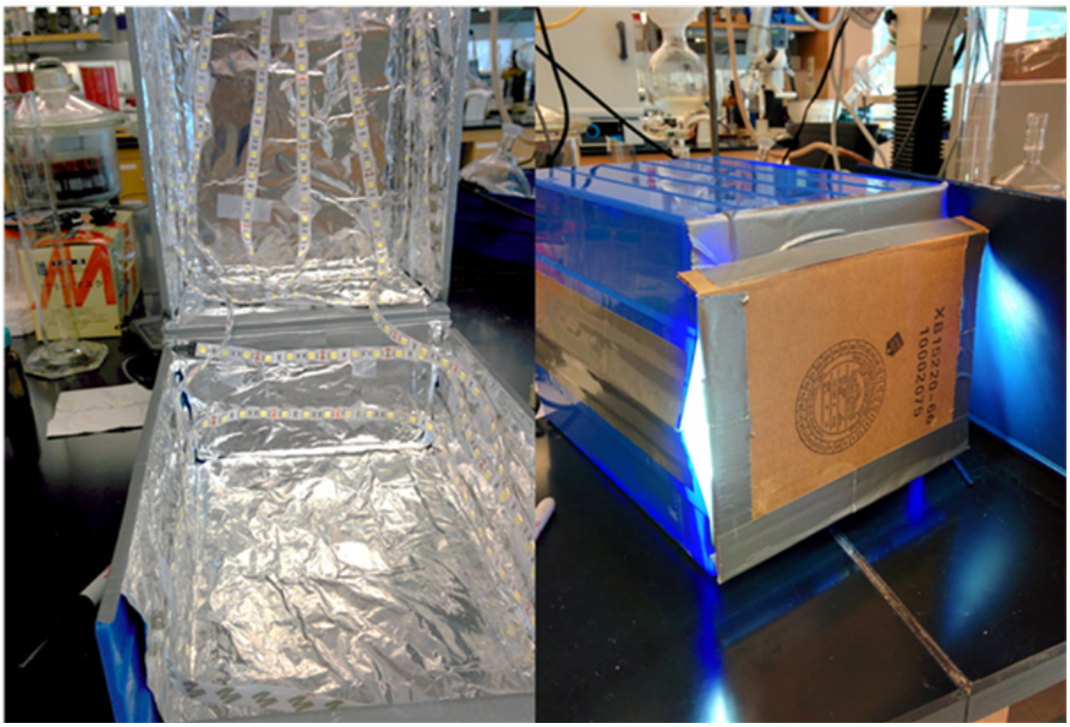


Figure S2. Experimental set-up for irradiation of **1**-SP/MCH$^+$ samples. A strand of broad-spectrum white LED lights taped along the foil-lined top and sides of two polypropylene bins duct-taped together with a foil-lined cardboard opening. A small hot-plate/oil bath was placed inside for temperature-controlled experiments.

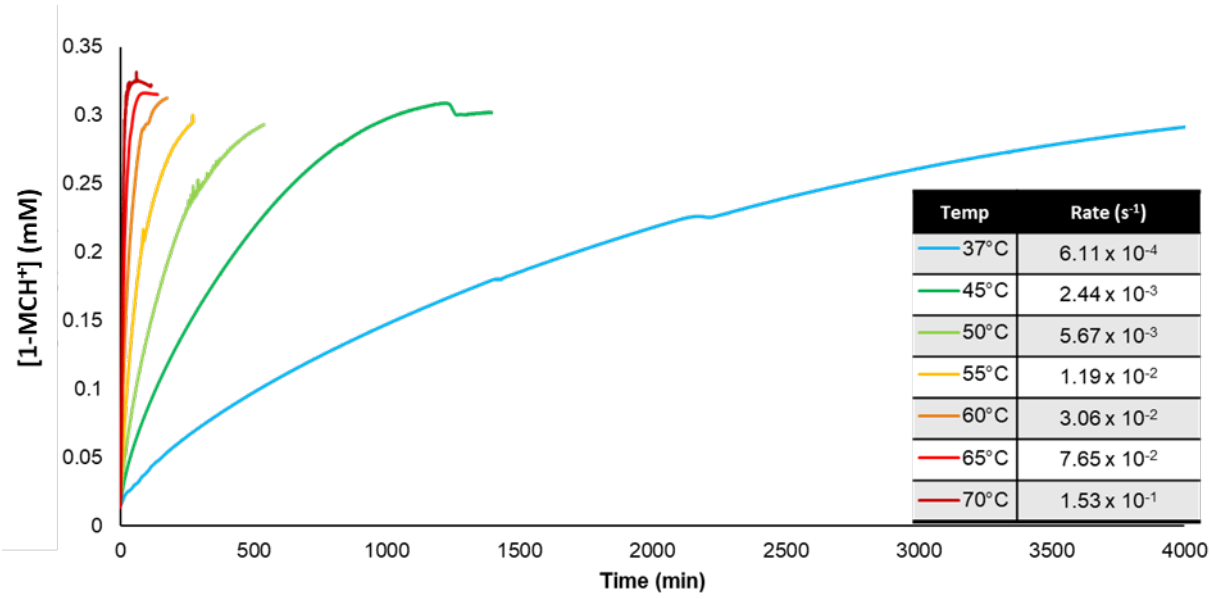


Figure S3. Plots of [**1**-MCH$^+$] versus time for **1**-SP in water (0.3 mM, 90 mM TFA) at various temperatures. Concentration was determined by monitoring the **1**-MCH$^+$ absorbance at 415 nm and converting the data to concentration using a standard curve. Rate constants were measured by fitting the change in [SP] concentration to a first order rate equation as described in the general methods.

Exp	Temp. (°C)	Rate constant (s^{-1})	$T_{>90\%}$
1	37	6.11×10^{-4}	~3 days
2	45	2.44×10^{-3}	20 h
3	50	5.67×10^{-3}	8 h
4	55	1.19×10^{-2}	4 h
5	60	3.06×10^{-2}	80 min
6	65	7.65×10^{-2}	60 min
7	70	1.53×10^{-1}	20 min

Exp	[TFA] (mM)	Rate constant (s^{-1})	$T_{>90\%}$
8	90	8.21×10^{-2}	60 min
9	45	1.12×10^{-1}	30 min
10	13	4.08×10^{-1}	15 min
11	180	2.15×10^{-2}	100 min
12	0.3	N/A	N/A

Table S1. Rate constants for the isomerization of **1**-SP based at different temperatures at constant TFA concentration (90 mM, Exps. 1-7) and using varying TFA concentrations at constant temperature (60°C, Exps. 8-13).

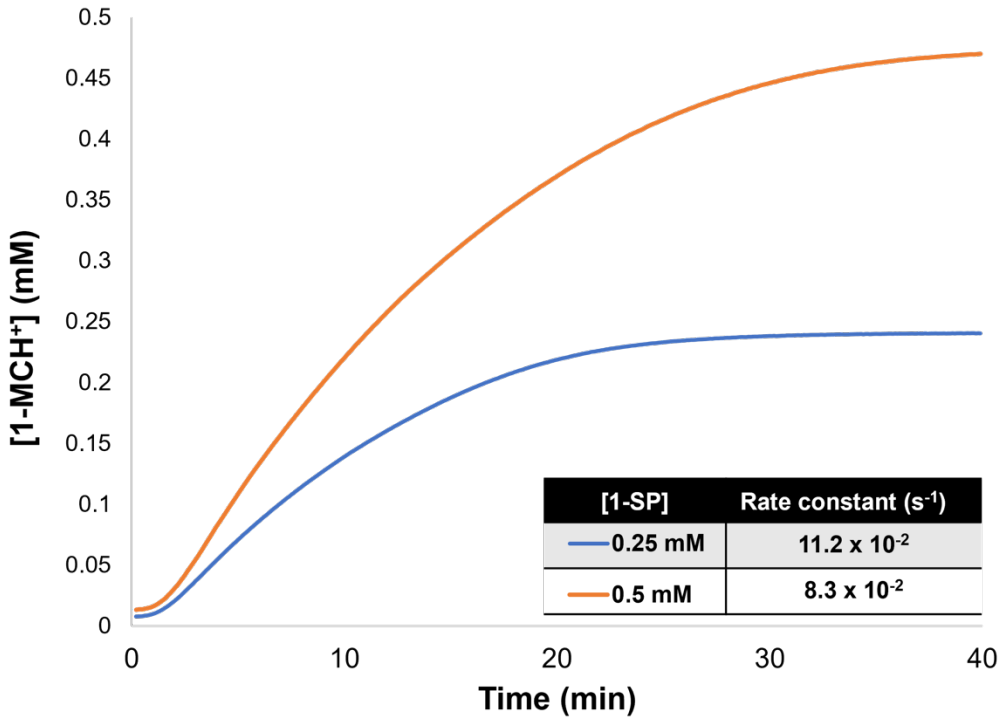


Figure S4. Plots of [**1**-MCH$^+$] versus time for **1**-SP in water (65°C, 90 mM TFA) at 2 different concentrations. Concentration was determined by monitoring the **1**-MCH$^+$ absorbance at 415 nm and converting the data to concentration using a standard curve. Rate constants were measured by fitting the change in [SP] concentration to a first order rate equation as described in the general methods.

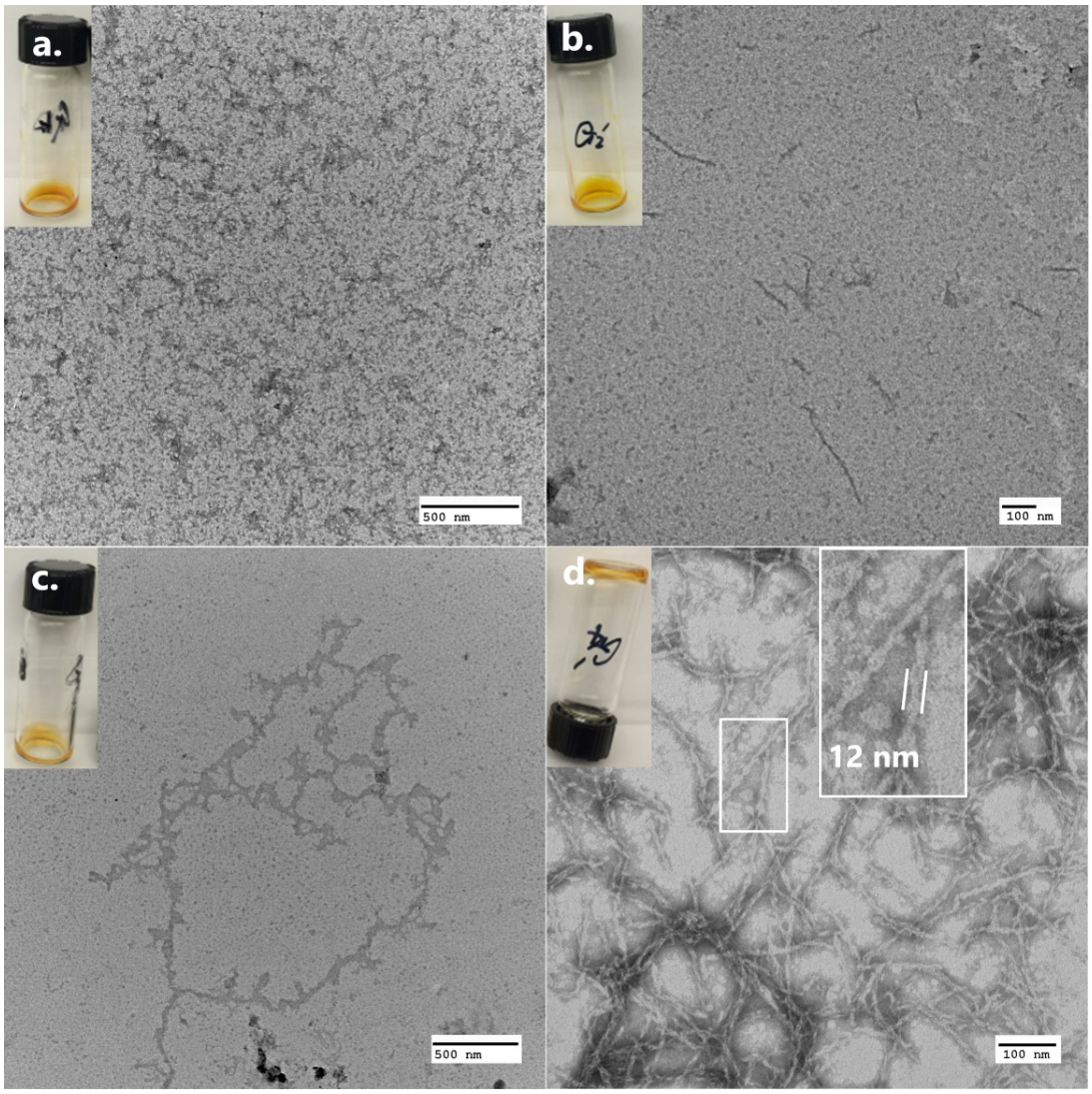


Figure S5. TEM images of aqueous solutions of **1**-SP (10 mM) under varying conditions. The solutions were heated to 60°C for 1 h to isomerize **1**-SP to **1**-MCH^+, then incubated for 72 h at 22°C. A) in pure H_2O (10 mM) under dark conditions (**1**-MC); B) in aqueous TFA (90 mM) under dark conditions (**1**-MCH^+); C) in pure H_2O under visible light (**1**-SP), and D) in aqueous TFA (90 mM) under visible light (**1**-SP). Samples for TEM were prepared by diluting to 1 mM prior to imaging. Additional details about TEM experimental procedures are located above.

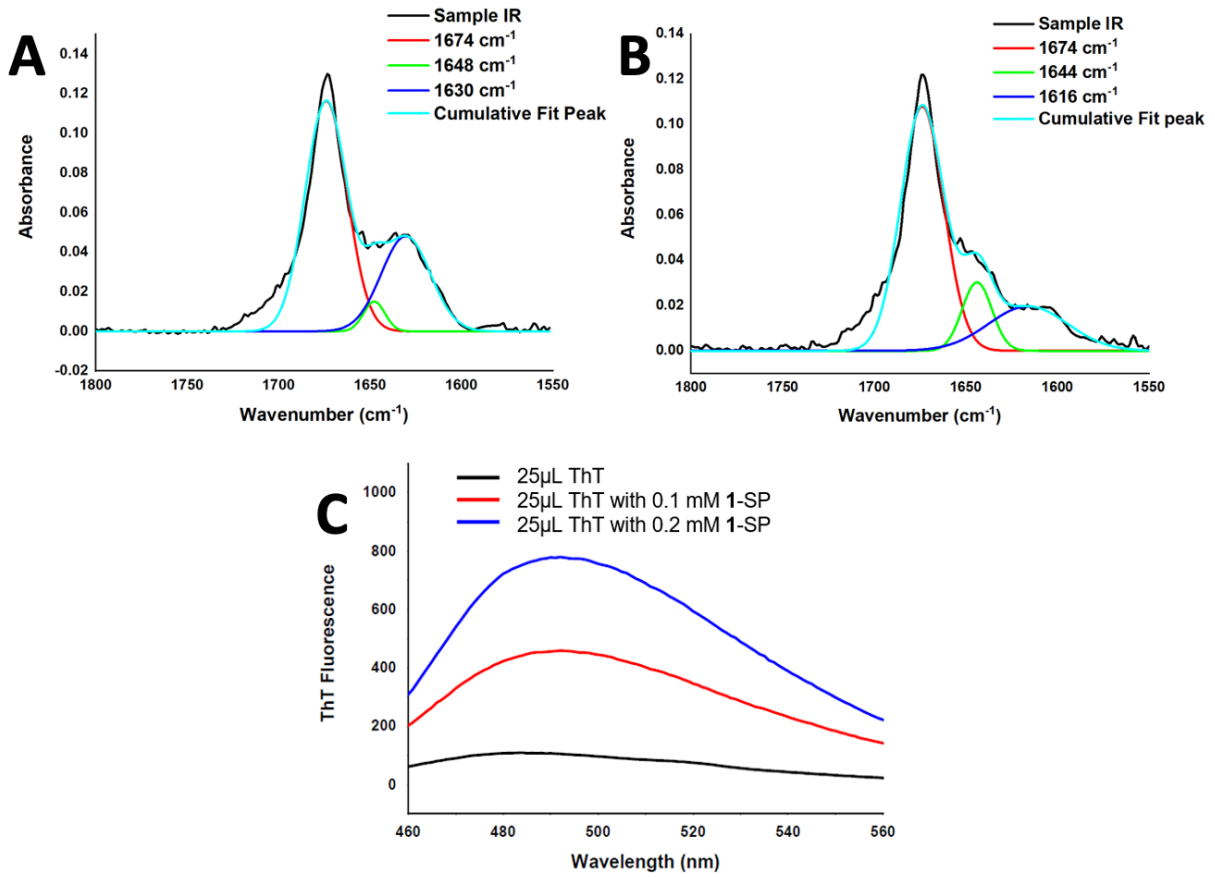


Figure S6. A) FT-IR spectra of a hydrogel of **1**-SP formed at a concentration of 20 mM in water (90 mM TFA) with visible light irradiation at 22°C over 3 days. The gel was lyophilized then redispersed in D_2O (20 mM) prior to recording the spectra. B) FT-IR spectrum of **1**-SP formed at a concentration of 20 mM in water (90 mM TFA) with visible light irradiation at 60°C for 1 h. The gel was lyophilized then redispersed in D_2O (20 mM) prior to recording the spectra. C) Fluorescence emission spectra of aqueous solutions of ThT (50 µM, PBS) with 0 (black), 1 (red) and 2 (blue) 5 µL aliquots of **1**-SP hydrogel (formed at 10 mM, 22°C over 7 days) dispersed in ThT solution. The observed increase in emission at ~490 nm (440 nm excitation) indicates the presence amyloid-type nanofibers in the hydrogel.

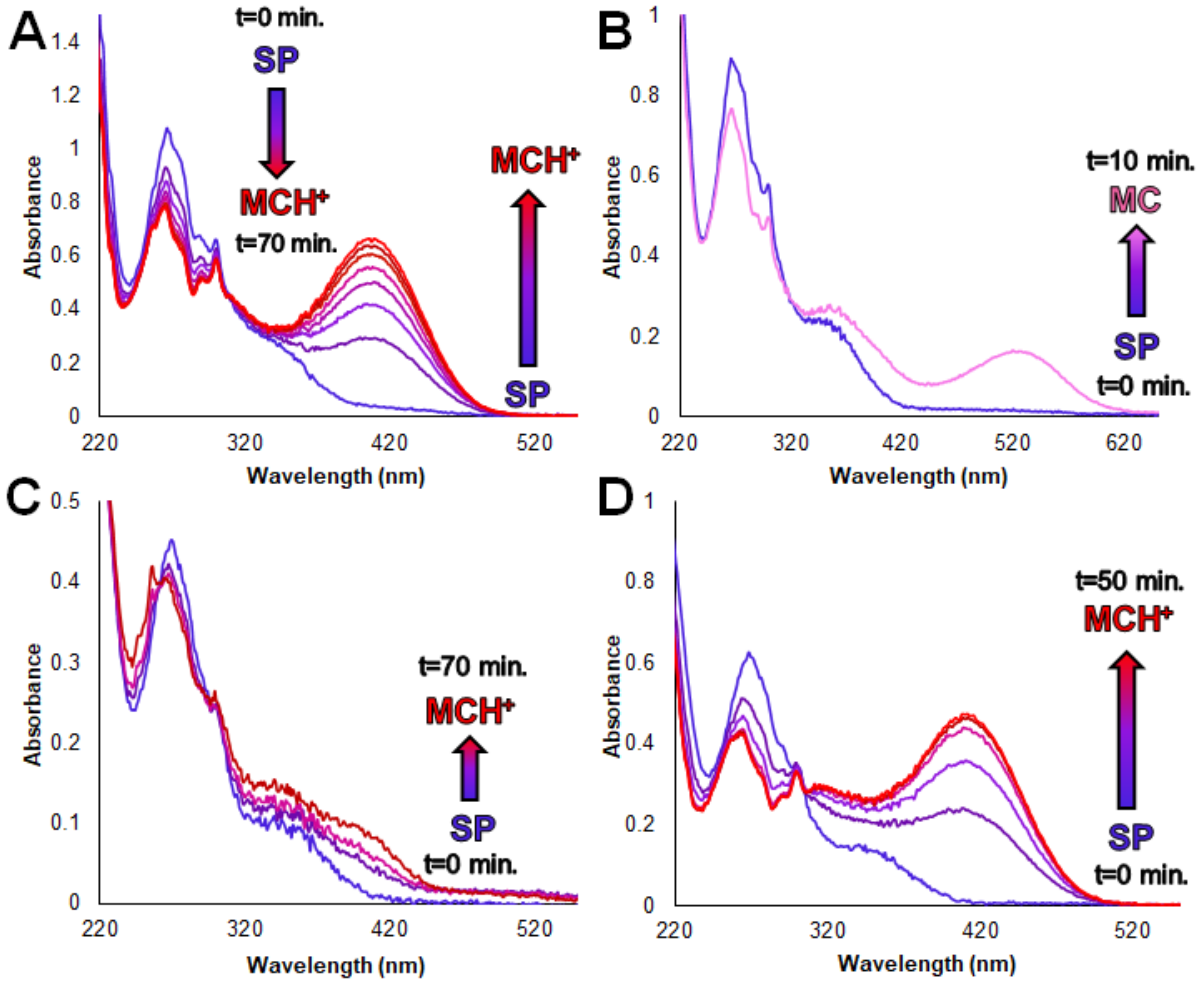


Figure S7. Thermal isomerization of **1**-SP/**1**-MCH$^+$. UV-Vis spectra showing 0.3 mM solutions of **1-SP** at 60°C/darkness A) in CH_3CN (90 mM TFA) showing an increasing concentration of **1**-MCH$^+$ over 70 min, B) in pure water without added TFA, showing formation of small amounts of the unprotonated merocyanine form (**1**-MC) (purple to pink); C) in water with added NaCl (90 mM) showing little change over 120 min, and D) water/90 mM HCl showing conversion to **1**-MCH$^+$ over 50 min.

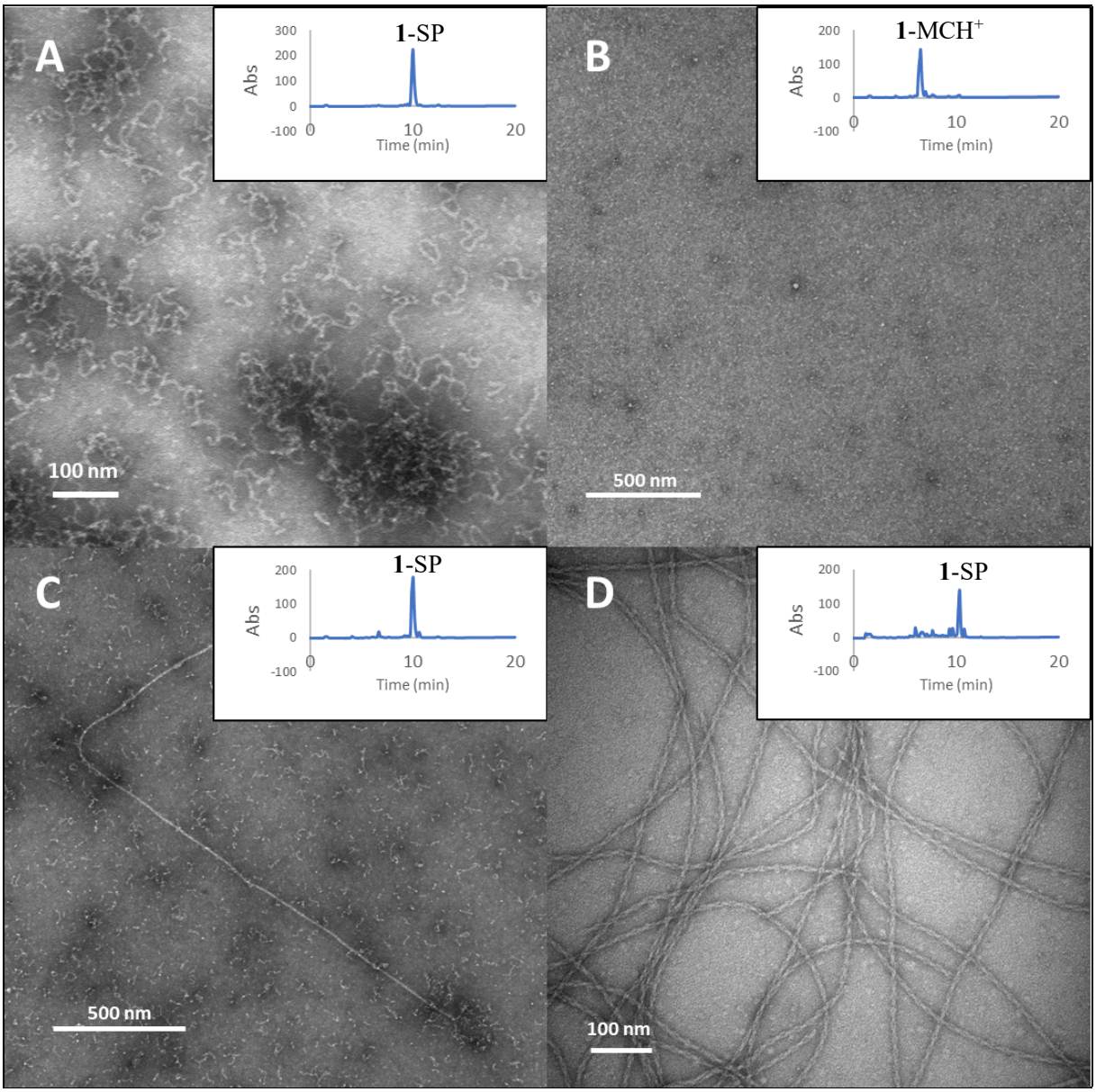


Figure S8. TEM images of **1**-SP assembled in water (3 mM, 90 mM TFA, stained with uranyl acetate) at various time points. A) immediately after dissolution, showing initial aggregates, B) after 60 min at 60°C in the dark, showing the lack of any aggregates formed characteristic of **1**-MCH^+, C) after 30 min at 60°C with visible light irradiation showing longer fibers beginning to form, and D) after 24 h at 60°C with visible light irradiation showing a dense network of nanofibers. Graphical inserts show analytical reverse-phase HPLC traces (C8 reverse-phase column using a CH_3CN/H_2O 0.1% TFA gradient) of the samples used for TEM imaging.

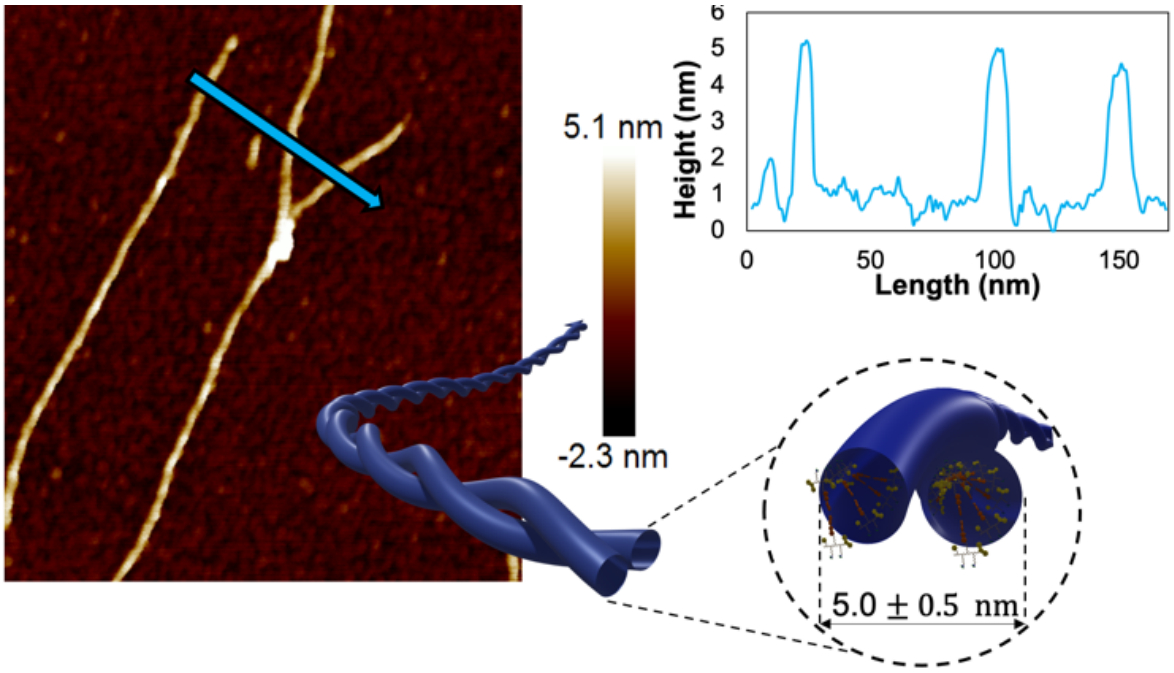


Figure S9. AFM images and height profiles of nanofibers of the hydrogel form by **1** form under dynamic conditions (60°C, visible light, 24 h).

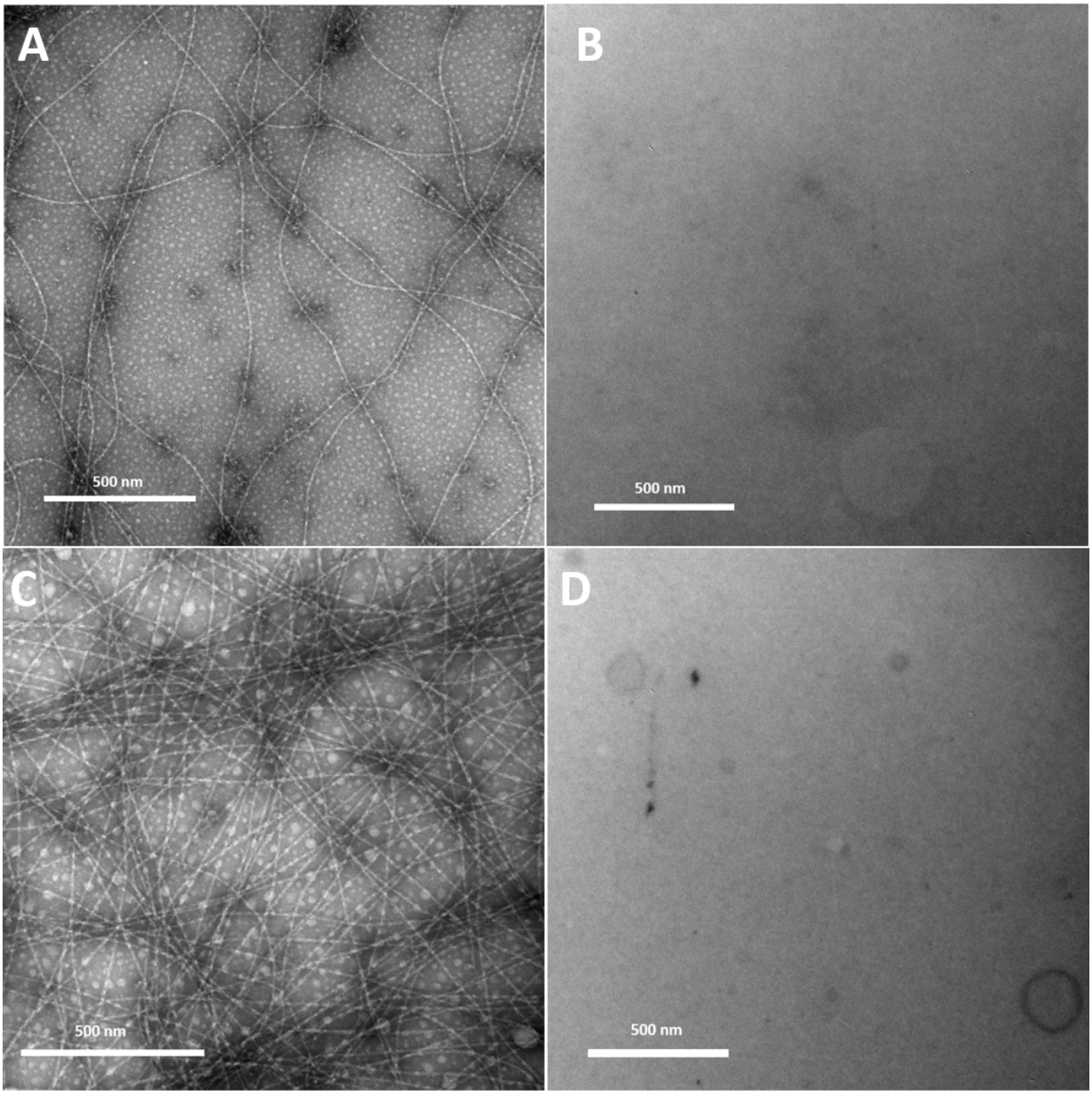


Figure S10. TEM images of **1**-SP (3 mM) at 60°C in 90 mM TFA during on/off cycling of visible light (Figure 4). A) t=2, 2 h of visible light, B) t=4, light source turned off for 2 h, C) t=6, 2 h of visible light, D) t=8, light source turned off for 2 h.

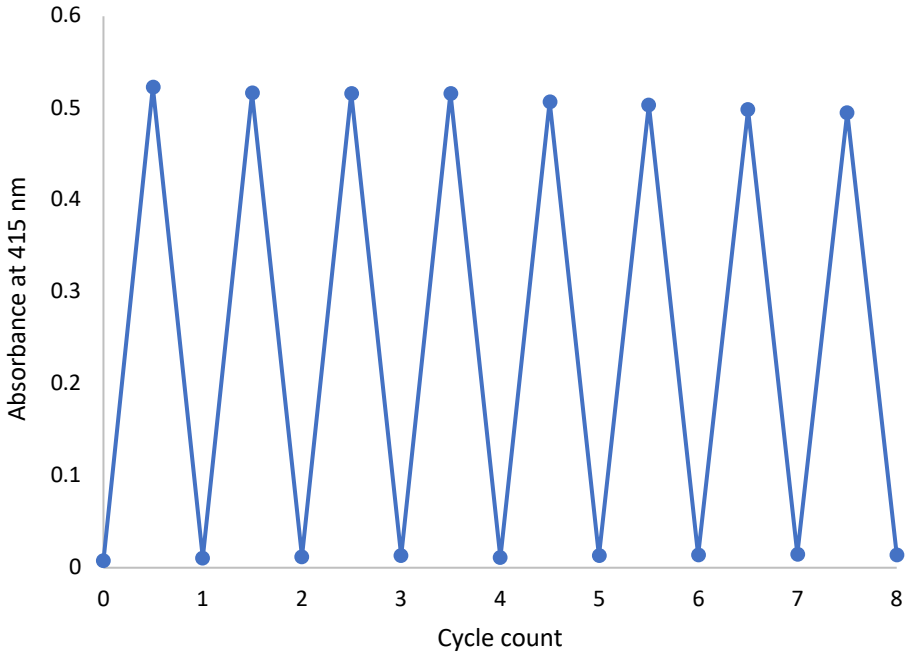


Figure S11. Photoswitching fatigue test on **1**-SP (0.3 mM) in 90 mM TFA in water. One cycle consists of 60 min. of heating in dark at 60°C, followed by 5 minutes of visible light irradiation. A decrease of 4.7% in the maximum absorbance at 415 nm exhibited by the solution was observed after 8 cycles.

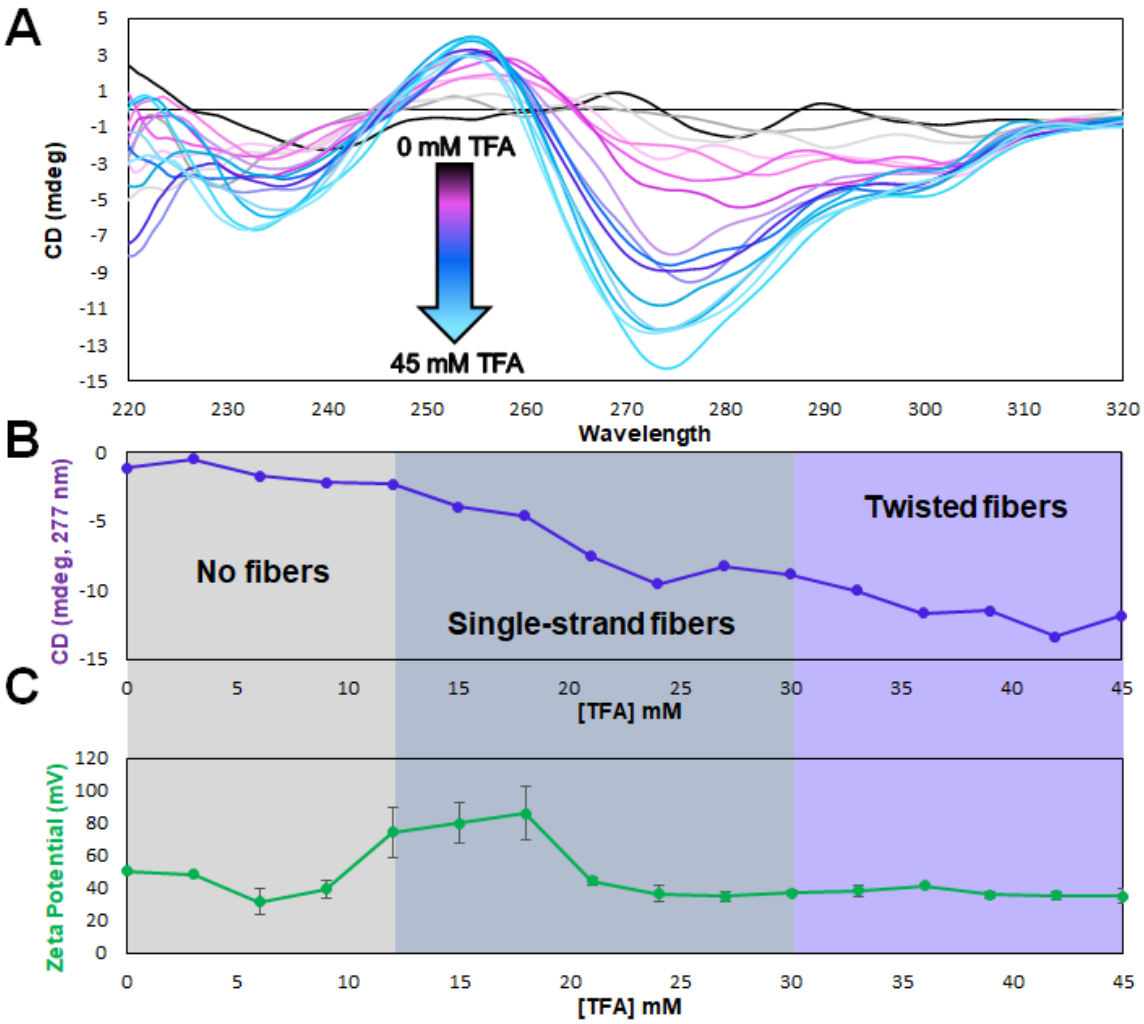


Figure S12. A) Self-assembly of 1 under dynamic conditions (4 h, 60°C, visible light, 3 mM **1**) as a function of TFA concentration (0- 45 mM) showing an increase in the CD signal as the concentration of TFA increases; B) CD signal at 277 nm plotted against TFA concentration. C) Zeta potential (mV) as function of TFA concentration.

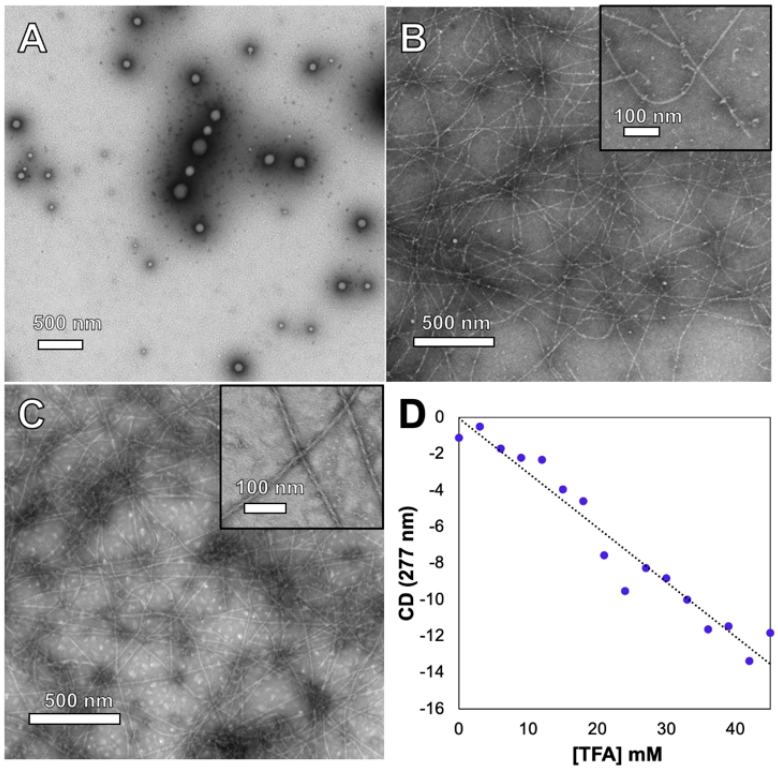
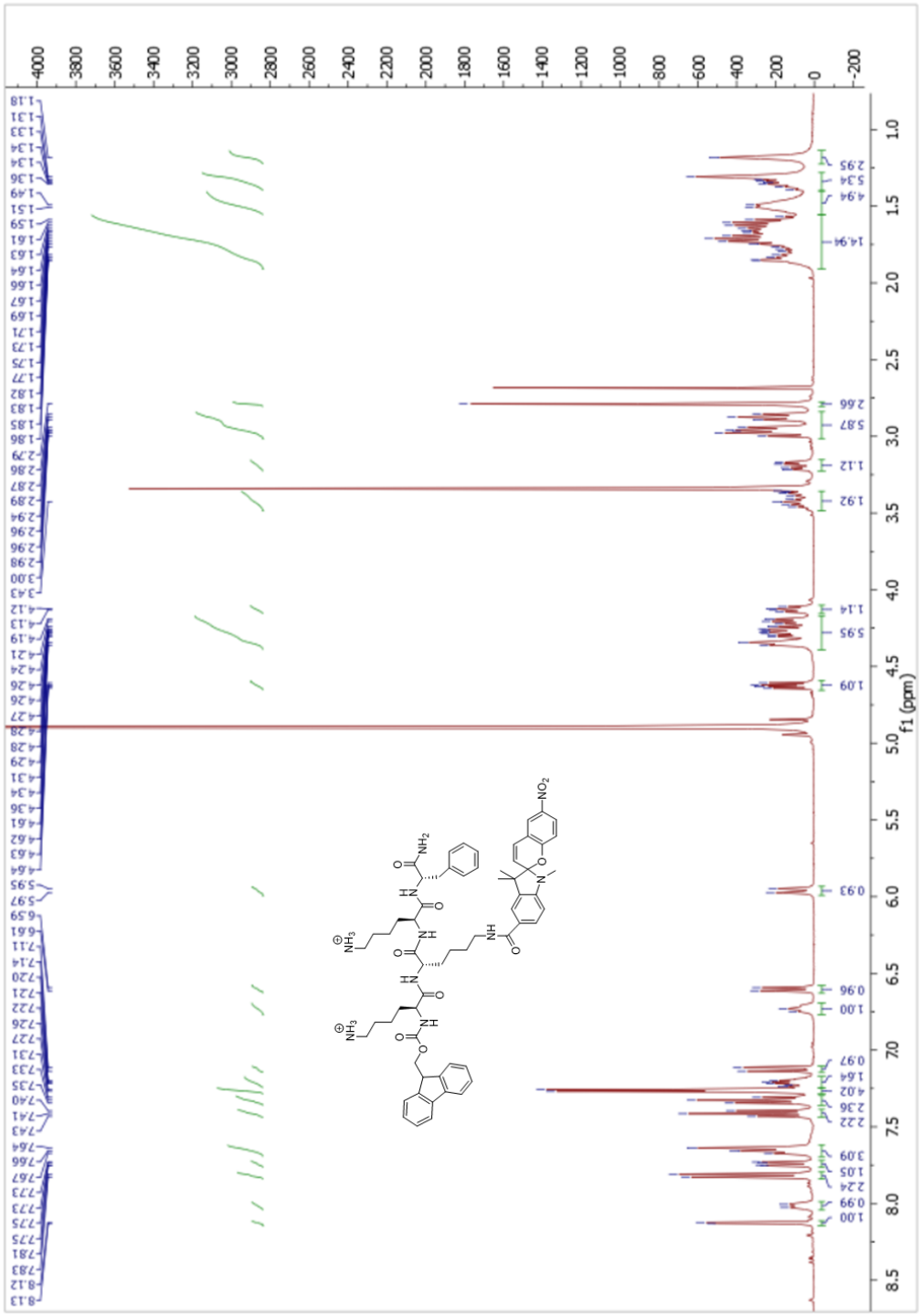
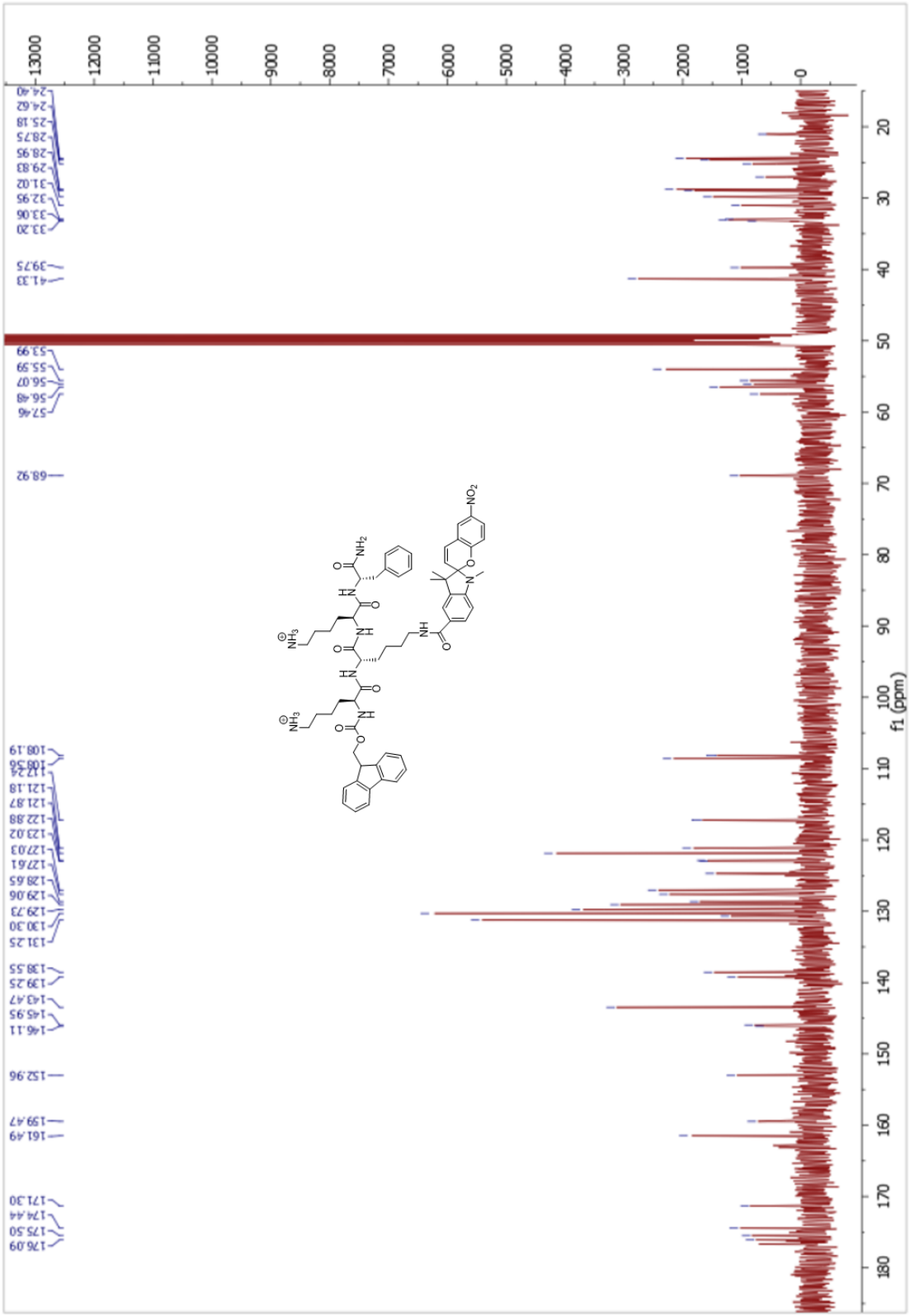


Figure S13. TEM images of **1**-SP (3 mM) in A) pure water, B) 13 mM TFA, and C) 90 mM NaTFA. D) Plot of CD signal at 277 nm of **1**-SP in water (3 mM) as function of TFA concentration, indicating progressively enhanced assembly. Samples of **1**-SP for each experiment were prepared under the dynamic self-assembly conditions (3 mM, 60°C, visible light).

Supporting Information

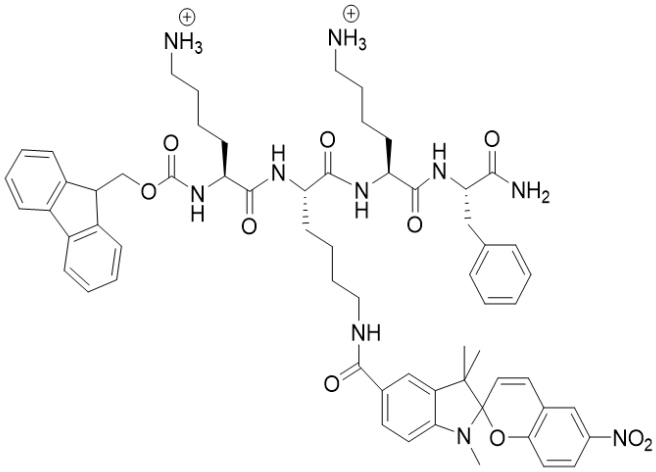
NMR Spectra

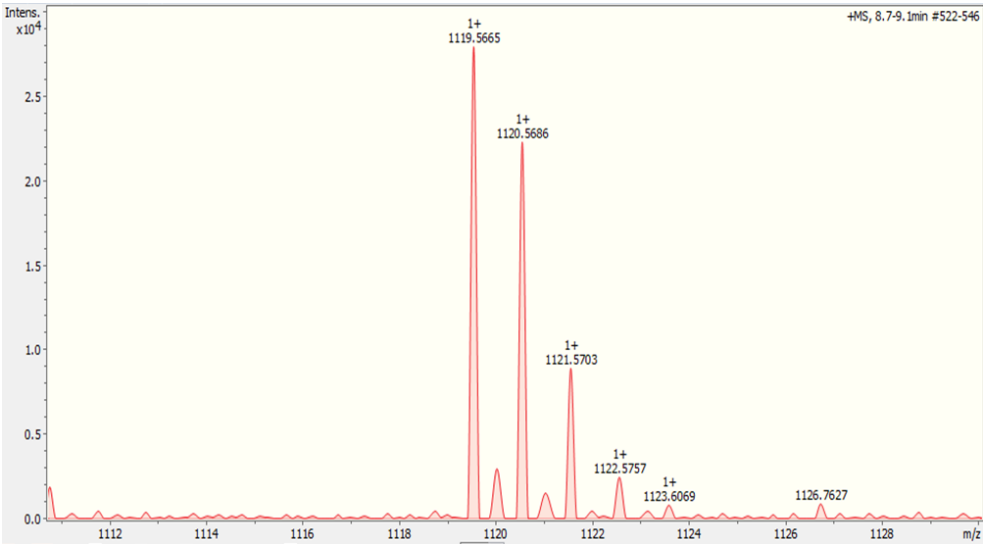



f1 (ppm)

Supporting Information

ESI-MS Analysis





References

1. Tomasulo, M.; Kaanumal, S. L.; Sortino, S.; Raymo, F. M. Synthesis and Properties of Benzophenone−Spiropyran and Naphthalene−Spiropyran Conjugates. *J. Org. Chem.,* **2007**, *72* (2), 595–605
2. Murugan, N. A.; Chakrabarti, S.; Agren, H. *Solid-Phase Peptide Synthesis*; 2011; Vol. 289.