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SUPPLEMENTARY INFORMATION

Photo-Crosslinking of a Self-Assembled Coumarin-Dipeptide Hydrogel

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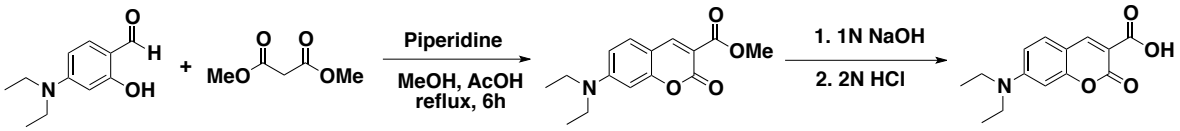
General Methods

Fourier transform-infrared (FT-IR) was performed on FT-IR spectrometer (Thermo Nicolet, Madison, WI). Circular dichroism (CD) spectra were taken with a Jasco CD spectrometer. Transmission Electron Microscopy (TEM) was performed with Technai G2 Spirit instrument operating at 80 kV. All reactions were performed under an argon or nitrogen atmosphere. ^{1}H NMR was recorded at 400 MHz and ^{13}C NMR spectra at 100 MHz on a Bruker DPX-400 instrument. Water (pH 7.0), 2,2,2-trifluoroethanol (TFE), and methanol used for CD, UV, and TEM were of spectroscopic grade, and phosphate buffered saline (PBS, pH 7.4) was purchased from Gibco.

Synthesis of DAC-CO_2H[1]

A solution of 4-diethylaminosalicylaldehyde (193.25 mg, 1 mmol) and dimethyl malonate (158.5 mg, 1.2 mmol) in MeOH (1 mL) was prepared. Piperidine (0.01 mM, 0.1 mmol) with catalytic amount of glacial acetic acid (1 drop) was added to the reaction mixture. After 3 h reflux, 2 mL H_2O was added to the mixture and cooled to 0 °C. NaOH (1N, aq) solution was added to the reaction mixture to form the yellow solid. The reaction mixture was refluxed for 20 min with vigorous stirring. After cooling to r.t., 2N HCl (aq) was added in order to acidify the solution. The orange crude solid was collected by filtration, washed with 50% cold ethanol (10 mL), and recrystallized with ethanol to obtain the bright orange crystalline product (206 mg, yield = 79 %). ^{1}H NMR(400 MHz, $CDCl_3$) δ 8.63 (1 H, s), 7.45 (1 H, d, J = 9.0), 6.73 (1 H, s), 6.52 (1 H, s), 3.50 (4 H, q, J = 7.1), 1.27 (7 H, t, J= 7.1); ^{13}C NMR

(100 MHz, $CDCl_3$) δ 165.56, 164.52, 158.05, 153.81, 150.26, 131.98, 110.97, 108.55, 105.44, 96.82, 45.37, 12.40; ESI-MS calcd for $C_{14}H_{15}NO_4$ $[M]^+$ 262.1079, found 262.1042.

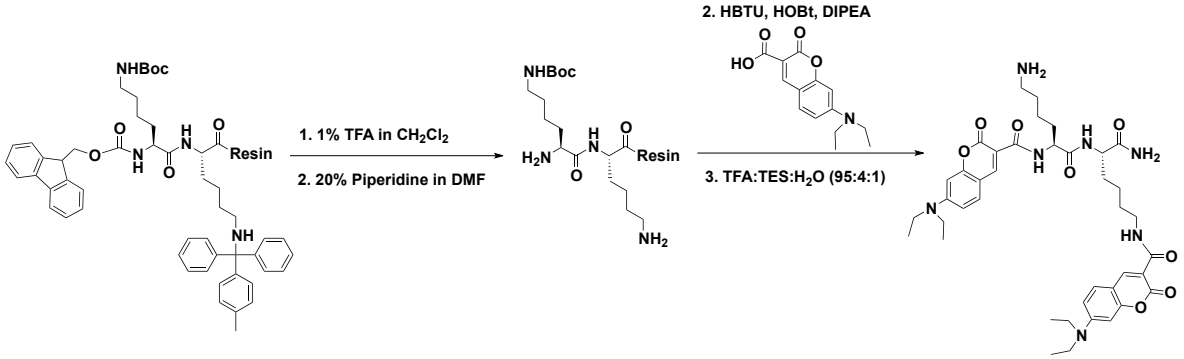


Scheme S1. Synthesis of coumarin carboxylic acid (7-(diethylamino)-2-oxo-2*H*-chromene-3- carboxylic acid, DAC-CO_2H).

Synthesis of DAC-KK(DAC)-NH_2. (Compound A):

Compound **A** was prepared *via* on-resin modification of the side chain (Scheme S2). The dilysine protected with Fmoc and Mtt protection groups was manually prepared using Fmoc/t-Bu solid-phase peptide synthesis on rink amide resin (loading 0.59 mmol/g). Amide-coupling steps were accomplished with standard techniques for all amino acids: Fmoc-amino acid, 1,3-diisopropylcarbodiimide (DIC), and 1-hydroxybenzotriazole (HOBt) (500 mol% each relative to resin) in 1:1 DMF/DCM for 1.5 h. A solution of 20 % piperidine in DMF was used for Fmoc removal and 1 % TFA in dichloromethane was used for Mtt group deprotection. A mixture of DAC-CO_2H, HBTU, HOBt, and DIPEA (300 mol% each relative to resin) in DMF was added to the resin. The reaction mixture was shaken for 24 h at room temperature and then filtered through a fritted syringe. The resin was washed thoroughly (3 x DMF, 3 x CH_2Cl_2) and the final DAC-peptide conjugate was cleaved from the resin by the treatment with TFA/water/triethylsilane (95 / 1 / 4) at room temperature for 2 h. The crude peptides were precipitated with cold diethyl ether and purified by reversed-phased HPLC on preparative Varian Dynamax C18 column eluting with a linear gradient of CH_3CN/water containing 0.1 % TFA (10/90 to 100/0 over 30 minutes) and stored as lyophilized powers at 0 °C. Peptide purity was assessed by analytical reverse-phase HPLC, and identity was confirmed using ESI-TOF mass spectrometry and NMR.

DAC-KK(DAC)-NH_2 ^{1}H NMR (400 MHz, DMSO-d^6) δ 9.03 (d, *J* = 7.6 Hz), 8.65-8.61 (m), 8.17 (d, *J* = 7.9 Hz), 7.69-7.61 (m), 7.40 (dd, *J* = 0.7, 0.4 Hz), 7.01-7.00 (m), 6.80 (dt, *J* = 9.1, 2.3 Hz), 6.61 (dd, *J* = 10.3, 2.2 Hz), 4.59-4.58 (m), 4.21-4.19 (m), 3.54-3.44 (m), 3.30-3.24 (m), 2.79-2.74 (m), 1.76-1.66 (m), 1.58-1.48 (m), 1.38-1.30 (m), 1.15-1.10 (m); ^{13}C NMR (100 MHz, DMSO-d^6) δ 173.55, 170. 89, 162.05, 161.78, 161.72, 161.69, 157.81, 157.27, 157.14, 152.51, 152.35, 147.78, 147.55, 131.61, 131.54, 131.49, 110.14, 110.12, 110.07, 109.41, 108. 98, 107.61, 107.60, 95.81, 52.33, 44.30, 38.77, 38.69, 32.27, 31.49, 28.83, 26.74, 22.93, 21.69, 12.29.; ESI-MS calcd for $C_{40}H_{53}N_7O_8$ $[M+H]^+$ 760.4034, found 760.4075.



Scheme S1. Synthesis of DAC-KK(DAC)-NH_2 (**A**).

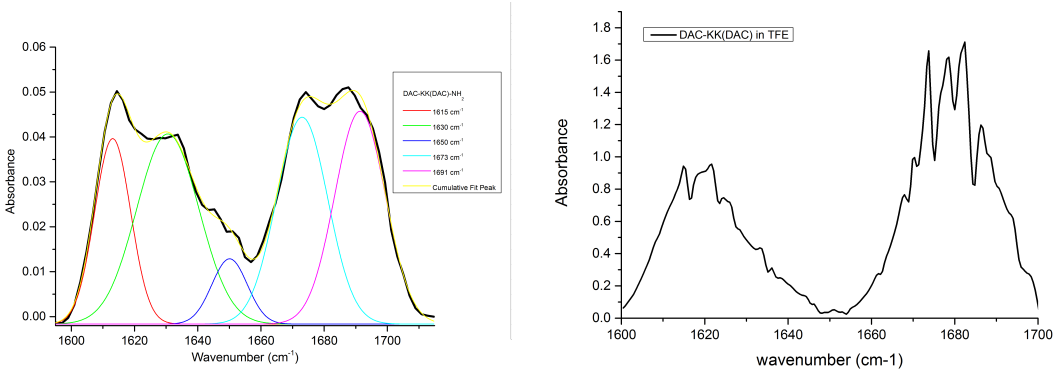


Figure S1. Left: Deconvolution of FT-IR spectrum of DAC-KK(DAC)-NH_2 (**A**). The solution was prepared in PBS (10 mM), lyophilized for 3 days to eliminate the water, and redissolved in D_2O. Right: FT-IR spectrum of DAC-KK(DAC)-NH_2 (**A**) dissolved in trifluoroethanol (10 mM). Dipeptide **A** remains monomolecular in TFE, which is reflected by the absence of the peak at 1630 cm^{-1}.

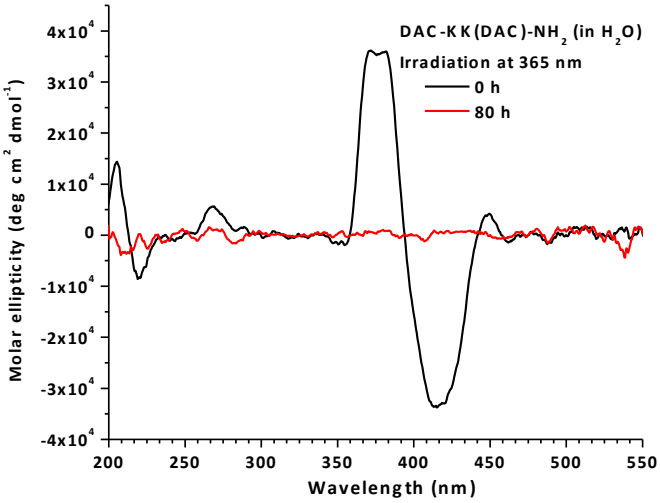


Figure S2. CD spectra of DAC-KK(DAC)-NH_2 (**A**) in water with and without photo-crosslinking. Samples (250 μM) were irradiated at 365 nm with UV lamp (6 W/ 0.16 Amps) for 80 h.

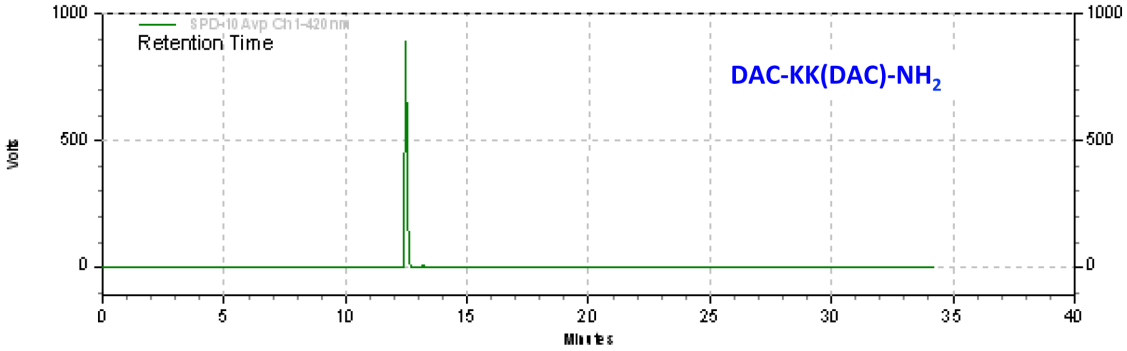


Figure S3. HPLC trace of purified DAC-KK(DAC)-NH_2 (**A**).

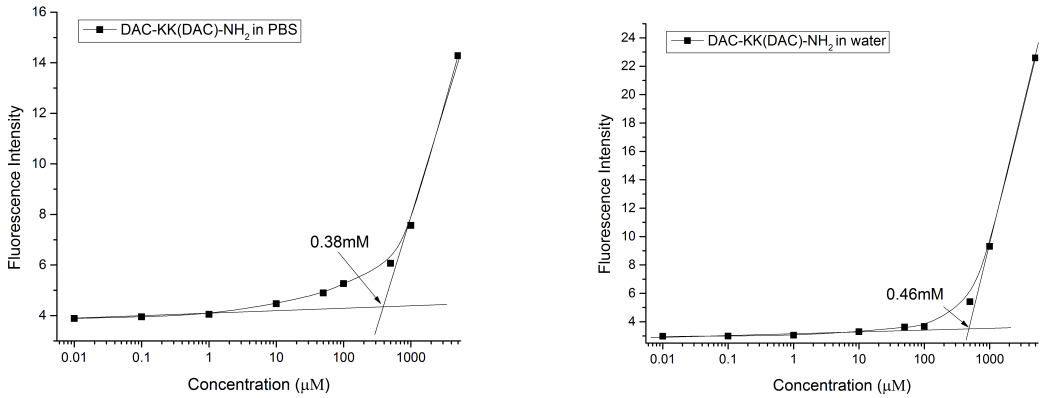


Figure S4. Plot of fluorescence intensity of Nile Red at 656nm (Ex = 550 nm) versus the concentration (mM) of DAC-KK(DAC)-NH_2(**A**) in PBS buffer and in pure water.

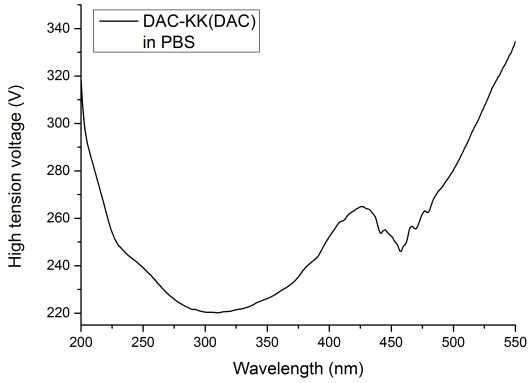


Figure S5. High Tension (HT) voltage spectra of DAC-KK(DAC)-NH_2 (**A**) in PBS. Samples was prepared from the PBS solution (10 mM) after 1day incubation at room temperature and freshly diluted to 0.25 mM before the measurement.

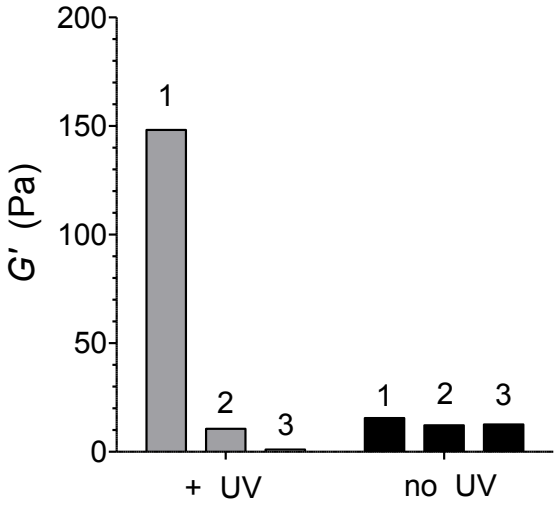


Figure S6. Elastic moduli (*G'*) of hydrogels prepared in pure water and subjected to three successive stress sweeps (labeled 1, 2, and 3) with (+ UV) and without (no UV) irradiation.

Circular Dichroism (CD) Spectroscopy Measurement.

CD spectra were recorded on a Jasco CD spectrometer under a nitrogen atmosphere. Experiments were performed in a quartz cell with a 1 mm path length over the range of 190-800 nm. Samples were prepared from both PBS and H_2O (5 mM for both) after 1 day incubation at room temperature, and subsequently diluted to 0.25 mM before the measurement.

UV-Vis Studies

Experiments were performed in a quartz cell with a 1 mm path length over the range of 190-800 nm. Samples were prepared from both PBS and H_2O (5 mM for both) after 1 day incubation at room temperature and freshly diluted into 0.25 mM before the measurement. Time dependent photocrosslinking was monitored by UV-Vis spectrophotometery as a function of UV irradiation duration (UVLS-26 EL series UV lamp, 6 W/0.15 A) at 365 nm. Photo-crosslinked polymers was collected by centrifugation and thoroughly washed with water. (10 mL × 3)

Fourier Transform Infrared (FT-IR) Spectroscopy Measurement and Deconvolution Procedure

All FT-IR spectra were collected on a Nicolet FTIR spectrometer at ambient temperature. The instrument was continuously purged with CO_2-free dry air. Spectra were recorded between 1700 and 1600 cm^{-1} at a resolution of 4 cm^{-1}, and a total of 64 scans were averaged. Samples for FT-IR were first dissolved in H_2O or PBS (5 mg/mL). A solution in PBS was freeze-dried to remove water and redissolve in D_2O. Spectra was analysed in a transmission cell having CaF_2 windows and a 0.025-μm path length. After subtracting the solvent spectra from the sample spectrum, the amide I band (1600-1700 cm^{-1}) of each spectrum was subjected to a fitting procedure using six Gaussian distribution (10 cm^{-1} full width at half maximum) centered at the frequencies based on Fourier self-deconvolution (FSD) spectra. During the fitting process, peak positions were constrained within limited intervals, whereas the peak heights were not constrained. The fitting "module" of the Origin software package was used for the fitting procedure. The contribution of each peak to the amide I band was quantified by the integrated areas of respective peaks.

Transmission Electron Microscopy Measurement – Negative Stain TEM

The dipeptide sample in PBS (5 mM, pH 7.4) and water (5 mM, pH 7.0) were prepared and aged for 1 day before the measurement. For the TEM microscopic studies, the samples were freshly diluted to 1 mM. Solutions (10 μL) in PBS and water were applied to carbon coated copper grid (Ted Pella, Inc.) for 2 min. After removing excess solution with filter paper, the grid was floated on 10 μL drops of 2 % wt uranyl acetate solution for negative staining for 1 min. Samples in water (0.25 mM) were irradiated at 365 nm for 4 days, and the insoluble precipitate was collected, thoroughly washed with water (10 mL × 3), and then collected again by centrifugation. This centrifuged polymer precipitate was resuspended in TFE. After 24 h incubation in TFE, 10 μL were aliquoted for TEM studies.

Critical aggregation concentration (CAC) measurements

The critical micelle concentration of DAC-KK(DAC)-NH_2 (**A**) was characterized by fluorescence spectra using Nile Red as the fluorescence probe. The sample was prepared together with Nile Red at different concentrations in PBS with the same concentration of Nile Red (20 μM). Fluorescence spectra were then recorded

at room temperature using a SHIMADZU RF-5301PC luminescence spectrometer. The excitation wavelength was set to 550 nm and the emission spectrum was recorded from 580 to 750 nm. Both excitation and emission slit widths were set to 5.0 nm. Upon the formation of micelle structures, the fluorescence intensity of Nile Red dramatically increased compared to non-encapsulated Nile Red, which would have a much lower fluorescence intensity. CAC values were calculated as the intersection of the tangents to the horizontal line of intensity ratio with relatively constant values and the diagonal line with rapidly increased intensity ratio.

Oscillatory shear testing

Compound **A** was prepared at 10 mM in pure HPLC-grade water, PBS (without Ca^{2+} and Mg^{2+}), or saline, and incubated at room temperature for 24 hours. Individual gel samples prepared in pure water were removed from their chambers by tilting them to delicately position the gel onto a flat spatula, and then loaded onto the rheometer plate. Gels prepared from PBS (w/ Ca^{2+}, Mg^{2+}) and saline were removed from vials first using a scalpel blade, and then a curved spatula, to disengage them from the glass walls and bottom, respectively. The complex shear storage (G') and loss (G'') moduli of these self-assembled hydrogels were then measured as a function of oscillatory stress using a rheometer (AR1000, TA Instruments, Delaware) equipped with either 40-mm aluminum parallel plate (gels in pure water) or 12-mm stainless steel parallel plate geometries. For photo-crosslinking gels in pure water, the gels were first prepared in humidified, sealed glass dishes for 24 h and subsequently exposed to UV light (λ = 365 nm) for 70 h before performing shear testing. Gels evolved from bright yellow to darker yellow with prolonged UV irradiation, while those exposed to UV >1 week collapsed into insoluble precipitates, as described previously.

1. Y. M. Ma, W. Luo, P. J. Quinn, Z. D. Liu and R. C. Hider, *J Med Chem*, 2004, 47, 6349-6362.