Supplementary Material (ESI) for Chemical Communications

Energy Transfer in Self-Assembled Dipeptide Hydrogels

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SUPPORTING INFORMATION

Experimental Details

Materials.

Anthrone, 2-naphthol, *iso*-butylchloroformate, *N*-methylmorpholine, phenylalanine ethyl ester, alanine ethyl ester, glucono-δ-lactone and lithium hydroxide were purchased from Sigma Aldrich and used as received. All solvents were purchased from Sigma Aldrich and used as received. Propyldansylamide was prepared as previously described.[1]

Synthesis of Gelators.

The naphthalene-diphenylalanine was prepared as described elsewhere for a related dipeptide. The final purity of the dipeptide as measured by NMR was > 98 %.

Ethyl 2-(2-(2-(naphthalen-2-yloxy)acetamido)-3-phenylpropanamido)-3-phenylpropanoate: ^{1}H NMR ($CDCl_3$) 7.79 (d, ArH, 2H, J = 8.4 Hz), 7.71 (d, ArH, 1H, J = 7.9 Hz), 7.47 (t, ArH, 1H, J = 6.2 Hz), 7.39 (t, ArH, 1H, J = 6.9 Hz), 7.25 (bs, NH, 1H), 7.16 – 7.02 (m, ArH, 11H), 6.97 (d, ArH, 1H, J = 6.7 Hz), 6.31 (d, NH, 1H, J = 6.8 Hz), 4.74 (m, C*H*NH, 2H), 4.53 (s, OCH_2, 2H), 4.14 (q, CH_2CH_3, 2H, J = 6.4 Hz), 3.13 – 2.94 (m, CH_2Ph, 4H), 1.27 (t, CH_2CH_3, 3H, J = 6.4 Hz) ppm. ^{13}C NMR ($CDCl_3$) 170.9, 169.8, 168.2, 154.9, 136.0, 135.6, 134.3, 129.9, 129.6, 129.3, 129.2, 128.7, 128.5, 127.7, 127.1, 127.1, 127.05, 126.7, 124.4, 118.1, 107.5, 67.1, 61.6, 53.8, 53.3, 37.9, 37.8, 14.1 ppm. MS (ES^+) 547 ($[M+Na]^+$). Accurate Mass calculated for $C_{32}H_{32}N_2O_5Na$: 547.2209. Found, 547.2216.

2-(2-(2-(Naphthalen-2-yloxy)acetamido)-3-phenylpropanamido)-3-phenylpropanoic acid: ^{1}H NMR (DMSO) 8.41 (d, NH, 1H, J = 7.8 Hz), 8.12 (d, NH, 1H, J = 8.5 Hz), 7.84 (d,

ArH, 2H, *J* = 7.2 Hz), 7.73 (d, ArH, 1H, *J* = 8.0 Hz), 7.45 (t, ArH, 1H, *J* = 8.0 Hz), 7.37 (t, ArH, 1H, *J* = 7.2 Hz), 7.24 – 7.15 (m, ArH, 12H), 4.64 (m, C*H*NH, 1H), 4.53 (s, OCH_2, 2H), 4.46 (m, C*H*NH, 1H), 3.11 – 2.84 (m, CH_2Ph, 4H) ppm. ^{13}C NMR (DMSO) 172.6, 170.7, 167.2, 155.4, 137.5, 137.4, 133.9, 129.3, 129.1, 129.0, 128.7, 127.9, 127.5, 126.8, 126.4, 126.2, 123.8, 117.4, 107.3, 66.6, 53.5, 53.2, 37.4, 36.7 ppm. MS (ES^+) 519 ($[M+Na]^+$). Accurate Mass calculated for $C_{30}H_{28}N_2O_5Na$: 519.1896. Found, 519.1912.

The anthracene-dialanine was prepared as follows:

To a stirred solution of anthrone (3.26 g, 16.8 mmol) and potassium carbonate (6.99 g, 3eq, 50.4 mmol), in acetone (125 mL), was added chloro-*tert*-butyl acetate (3.0 mL, 1.2eq, 20.2 mmol). The solution was wrapped in aluminium foil and heated to reflux overnight. After this time, the solution was filtered and the solvent removed *in vacuo*. The orange/yellow residue was dissolved in chloroform (ca. 100 mL), washed with distilled water (100 mL × 4), dried over anhydrous magnesium sulfate, filtered, and the solvent remove *in vacuo*. The orange residue was purified by flash column chromatography, eluting with 5% ethyl acetate in hexanes, to give ***tert*-butyl-2(anthracen-9-yloxy)acetate** as a yellow solid in a 53 % yield. ^1H NMR ($CDCl_3$) 8.39 (d, ArH, 2H, $^3J_{HH}$ = 8.6Hz), 8.26 (s, ArH, 1H), 7.99 (d, ArH, 2H, $^3J_{HH}$ = 7.5 Hz), 7.48 (m, ArH, 4H), 4.69 (s, OCH_2, 2H), 1.58 (s, $O(CH_3)_3$ 9H) ppm. ^{13}C NMR ($CDCl_3$) 168.6, 151.0, 132.7, 128.8, 126.0 (d, 5.74Hz), 124.8, 123.3, 122.7, 82.7, 73.2, 28.6 ppm. MS (CI+ (NH_3) 309 ($[MH]^+$), Accurate mass calculated: 309.14907. Found: 309.14828.

To a stirred solution of *tert*-butyl-2(anthracen-9-yloxy)acetate (6.40g, 20.75 mmol) dissolved in chloroform (100mL), was added trifluoroacetic acid (9.69 mL, 6.1eq, 0.13 mol). The solution was stirred for 48h. After this time, the solution was added *via* Pasteur pipette to a 500mL round bottomed flask containing hexane (ca. 400mL). The product was collected by filtration, washed with hexane and dried to give **2-(anthracen-9-yloxy)acetic acid** as a golden yellow powder in a 52 % yield. ^1H NMR (DMSO) 8.43 (s, ArH, 1H), 8.37 (m, ArH, 2H), 8.11 (m, ArH, 2H), 7.55 (m, ArH, 4H), 4.50 (s, OCH_2, 2H) ppm. ^{13}C NMR (DMSO) 174.5, 170.6, 150.7, 134.9, 133.4, 132.5, 132.2, 129.5, 128.7, 127.1, 126.1 (d, J = 2.8Hz), 125.9, 124.3, 123.0, 122.4, 72.3, 59.9 ppm. MS (CI+NH_3) 253 ($[MH]^+$), Accurate mass calculated: 253.08647. Found: 253.08596.

The amino acids were then sequentially coupled and deprotected as described elsewhere.

Ethyl 2-(2-(anthracen-9-yloxy)actamido)propanoate: Yield yellow crystals 73 %. ^{1}H NMR ($CDCl_3$) 8.28 (s, ArH, 1H), 8.19 (m, ArH, 2H), 8.01 (m, ArH, 2H), 7.83 (d, NH, 1H, J = 7.9 Hz), 7.49 (m, ArH, 4H), 4.88 (m, C*H*(CH_3)C=O, 1H), 4.70 (d, OC*H*$_2$, 1H, J = 15.2 Hz), 4.66 (d, OC*H*$_2$, 1H, J = 15.2 Hz), 4.31 (q, OC*H*$_2$CH$_3$, 2H, $^{3}J_{HH}$ = 7.1 Hz), 1.64 (d, (C*H*$_3$)CHC=O, 3H, $^{3}J_{HH}$ = 7.2 Hz), 1.36 (t, OCH$_2$C*H*$_3$, 3H, $^{3}J_{HH}$ = 7.1 Hz) ppm. ^{13}C NMR ($CDCl_3$) 173.1, 168.6, 149.2, 134.5, 132.7, 129.1, 127.6, 126.4, 126.3 (d, J = 35.39Hz), 124.6, 123.9, 121.8, 73.8, 62.1, 48.3, 19.1, 14.6 ppm. MS (ES+, CH_3OH), 374 ([M+Na]$^{+}$), accurate mass calculated: 374.1357. Found 374.1368.

2-(2-(Anthracen-9-yloxy)acetamido)propanoic acid: Yield yellow powder: 60%. ^{1}H NMR (DMSO) 8.74 (d, NH, 1H, $^{3}J_{HH}$ = 7.4 Hz), 8.44 (s, ArH, 1H), 8.35 (m, ArH, 2H), 8.12 (m, ArH, 2H), 7.56 (m, ArH, 4H), 4.68 (d, OC*H*, 1H, $^{3}J_{HH}$ = 13.8Hz), 4.59 (d, OC*H*, 1H, $^{3}J_{HH}$ = 13.8Hz), 4.45 (m, (CH$_3$)C*H*C=O, 1H), 3.36 (s, OH), 1.42 (d, C*H*$_3$, 3H, $^{3}J_{HH}$ = 7.4Hz) ppm. ^{13}C (DMSO) 167.8, 150.4, 135.0, 132.2, 128.8, 127.1, 126.2, 124.3, 123.1, 122.4, 74.3, 47.8, 17.5 ppm. MS (ES+, CH_3OH) 346 ([M+Na]$^{+}$), accurate mass calculated: 346.1042. Found: 346.1055.

Ethyl 2-(2-(2-(anthracen-9-yloxy)acetamido)propanamido)propanoate was prepared via coupling of 2-(2-(anthracen-9-yloxy)acetamido)propanoic acid with alanine ethyl ester as above. Due to the apparent sensitivity of this molecule to light, the crude mixture was directly deprotected using LiOH in THF/water as above.

2-(2-(2-(Anthracen-9-yloxy)acetamido)propanamido)propanoic acid: Yield yellow powder: 27 %. ^{1}H NMR 8.46 (d, NH, 1H, J_{HH} = 7.8 Hz), 8.44 (s, ArH, 1H), 8.34 (m, ArH, 2H), 8.10 (m, ArH, 2H), 7.54 (m, ArH, 4H), 4.66 (d, OCH, 1H, J_{HH} = 13.8 Hz), 4.62 (d, OCH, 1H, J_{HH} = 13.8 Hz), 4.56 (m, C*H*NH, 1H), 4.27 (m, C*H*NH, 1H), 1.35 (d, CH_3, 3H, J_{HH} = 7.1 Hz), 1.32 (d, CH_3, 3H, J_{HH} = 6.9 Hz) ppm; ^{13}C NMR 173.9, 171.7, 167.0, 149.9, 134.6, 133.0, 131.8, 128.4, 126.7, 125.8, 123.9, 122.7, 121.9, 73.9, 47.6, 47.4, 18.5, 17.1 ppm. MS (ES^{+}) 417 ([M+Na]$^{+}$). Accurate Mass calculated 417.1426. Found, 417.1437. The final purity by ^{1}H NMR was > 97 % %.

Hydrogel Formation

Naphthalene-diphenylalanine (25.0 mg, 0.051 mmol) was weighed into a sample tube. Water (5 mL) was added followed by sodium hydroxide (0.8 mL of a 0.1M aqueous solution) and

the solution stirred overnight. For the current studies, this solution was diluted by a factor of 4 for all experiments (final concentration 1.08 mg/mL, 2.2 mM). To induce gelation, glucono-δ-lactone (7.5 mg) was added to 2 mL of the peptide solution. The sample was swirled gently for 10 seconds to mix and then allowed to stand. A clear, self-supported gel formed over time.

For gels containing II or III, stock solutions of II (3.44 mM in methanol) and III (12.1 mM at pH 11 via addition of NaOH to a suspension of III in water as above) were prepared. 10 μL of either stock solution were added to a solution of I at high pH before GdL addition. To induce gelation, glucono-δ-lactone (7.5 mg) was added to 2 mL of the peptide solution. The sample was swirled gently for 10 seconds to mix and then allowed to stand. A clear, self-supported gel formed over time.

Instrumentation.

NMR. ^{1}H NMR spectra were recorded at 400.13 MHz using a Bruker Avance 400 NMR spectrometer. ^{13}C NMR spectra were recorded at 100.6 MHz.

Fluorescence Spectroscopy. Fluorescence spectra were obtained on a PerkinElmer Luminescence spectrometer LS55. The slit width for emission and excitation were 2.5 nm and the scan rate was 100 nm/min.

Transmission Electron microscopy. Samples for examination by TEM were prepared *in situ* on formvar/carbon film coated 400-mesh copper grids (Agar scientific). The required amount of GdL was added to a solution of I (0.5wt% solution at pH 10.7) and immediately grids were placed inverted onto 10 μl droplets of the gelation solution. In a humid chamber at room temperature, material was allowed to adsorb onto the grids and removed after 2 hours followed by 5 minutes of drying and two 1 minute negative stains using 2 % w/v uranyl acetate. Negatively stained grids were allowed to dry and examined on a Hitachi-7100 TEM operated at 100 kV. Images were acquired digitally using an axially mounted (2000 × 2000 pixels) Gatan Ultrascan 1000 CCD camera (Gatan, Oxford, UK).

Rheology. Dynamic rheological experiments were performed on an Anton Paar Physica MCR101 rheometer. In the oscillatory shear measurements, a sandblasted parallel top plate with a 50 mm diameter and 1.0 mm gap distance were used. Gels for rheological experiments were prepared on the bottom plate of the rheometer by loading a 2.0 mL solution of the gelator immediately after GdL addition. At this point, the sample is still a free-flowing liquid.

Hence, sample uniformity and reproducibility is high. Evaporation of water from the hydrogel was minimized by covering the sides of the plate with low viscosity mineral oil. The measurements of the shear modulus (storage modulus G′ and loss modulus G″) with gelation were made as a function of time at a frequency of 1.59 Hz (10 rad/s) and at a constant strain of 0.5 % for a period of 2 hours, followed by an amplitude sweep from 0.01 % to 100 % under a frequency of 10 rad/s. The strain amplitude measurements were performed within the linear viscoelastic region, where the storage modulus (G′) and loss modulus (G″) are independent of the strain amplitude. All the experiments were conducted at 25 ^{o}C.

pH measurements. A FC200 pH probe (HANNA instruments) with a (6 mm × 10 mm) conical tip was employed for all the pH-measurements. The stated accuracy of the pH measurements is ± 0.1. The pH changes during the gelation process were recorded every 1 minute for 24 hrs. All measurements were conducted at room temperature.

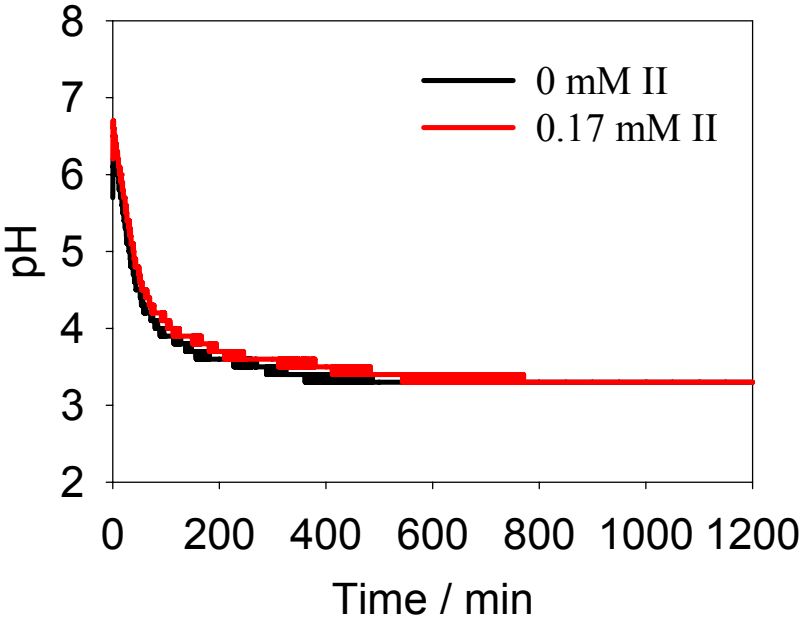

Figure S1. (Black data) Evolution of pH with time for a solution of dipeptide I (2.2 mM) on addition of GdL (3.75 mg/mL). (Red data) Evolution of pH with time for a solution of dipeptide I (2.2 mM) containing II (0.17 mM) on addition of GdL (3.75 mg/mL).

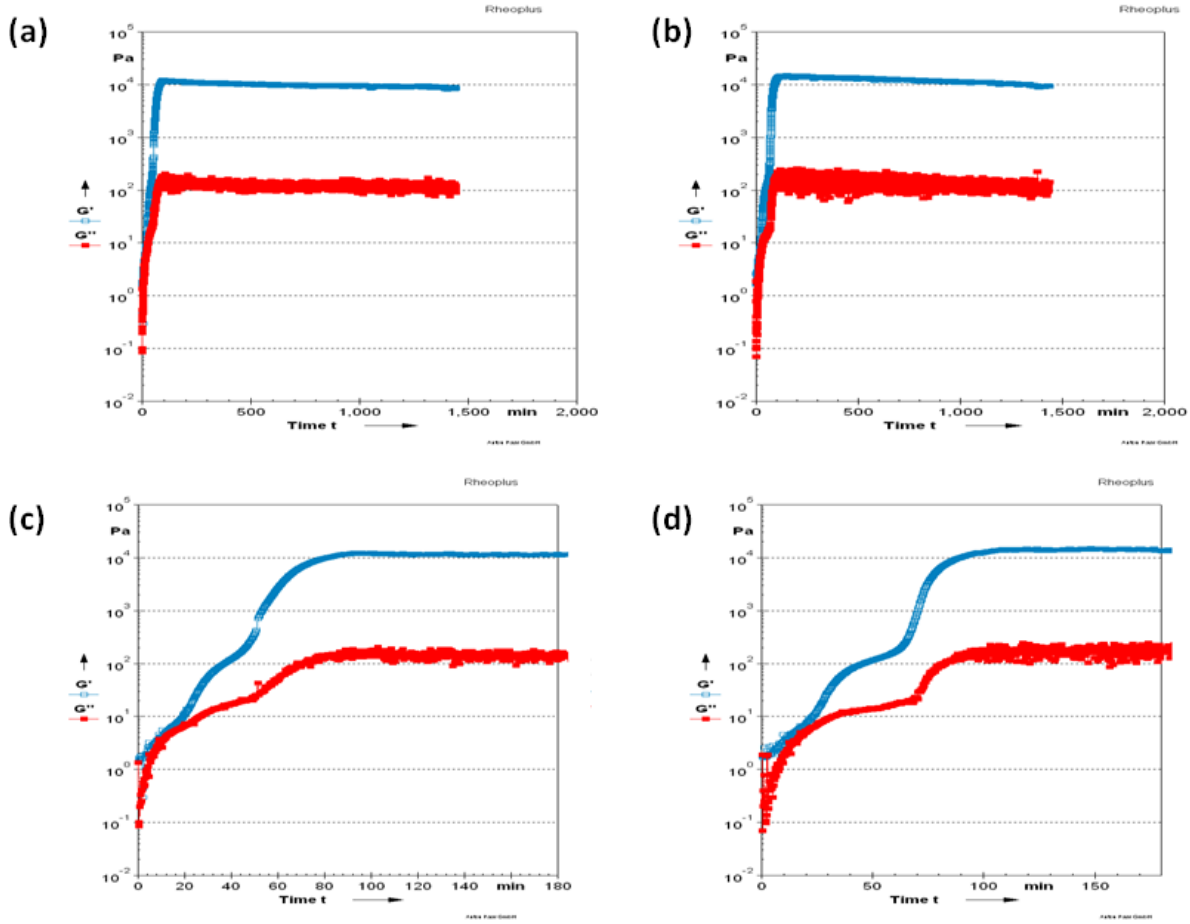

Figure S2. (a) Evolution of G′ and G″ with time for a solution of dipeptide I (2.2 mM) on addition of GdL (3.75 mg/mL). (b) Evolution of G′ and G″ with time for a solution of dipeptide I (2.2 mM) containing II (0.17 mM) on addition of GdL (3.75 mg/mL). (c) Expansion of (a) showing first 180 minutes. (d) Expansion of (b) showing first 180 minutes. In all cases, the blue data represents G' and red data G''.

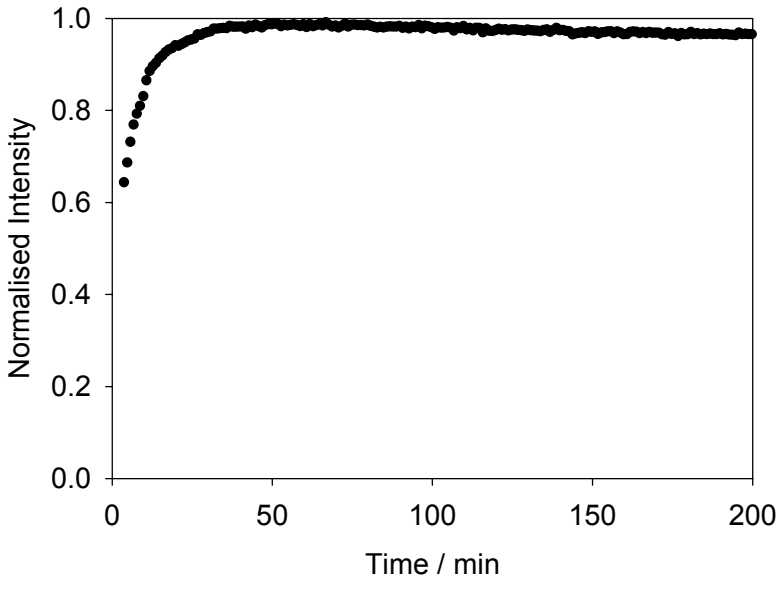

Figure S3. Normalized change in ThT fluorescence at 485 nm (λex = 455 nm) of solutions of dipeptide I (2.2 mM) on addition of GdL (3.75 mg/mL). (blue data). Protocols were as for those in reference 2. The assembly of the dipeptide-conjugate leads to incorporation of the ThT and hence an increase in the fluorescence from the ThT as described in reference 2. The timescale for assembly of the dipeptide-conjugate closely mirrors the evolution in rheological properties shown in Figure S2.

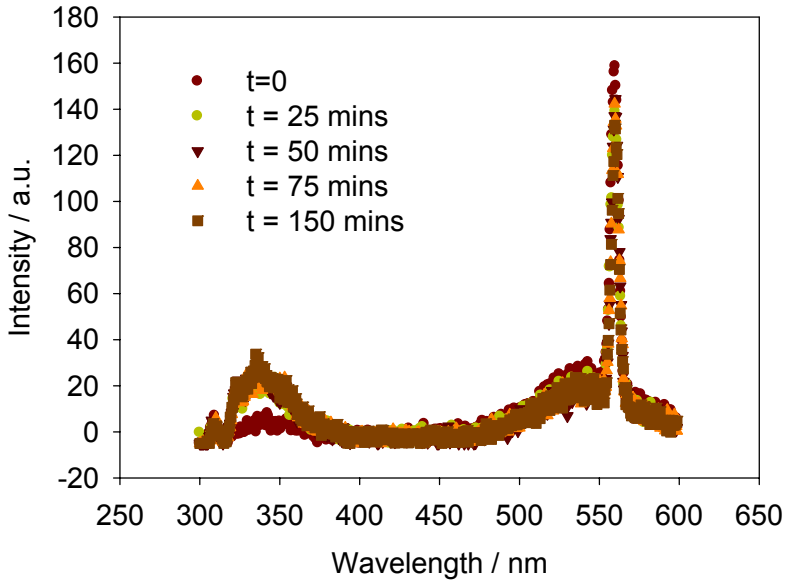

Figure S4. Evolution in emission from II alone (0.084 mM, excitation wavelength = 280 nm) on addition of GdL (3.75 mg/mL). As can be seen, no significant change in the emission is observed from II alone as the pH decreases. The sharp peak at 560 nm is due to Rayleigh scatter arising from poor excitation of II at this excitation.

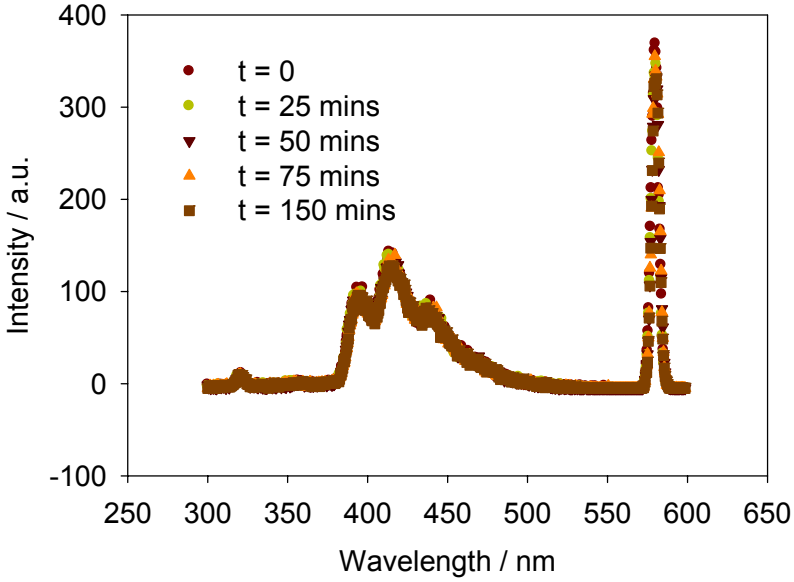

Figure S5. Evolution in emission from III (0.060 mM, excitation wavelength = 290 nm) alone on addition of GdL (3.75 mg/mL). As can be seen, no significant change in the emission is observed from III alone as the pH decreases. The sharp peak at 580 nm is due to Rayleigh scatter arising from poor excitation of III at this excitation.

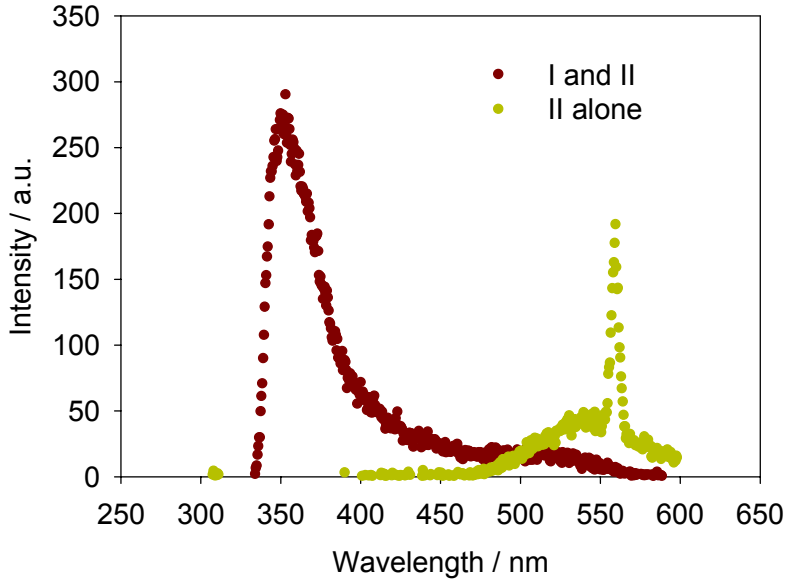

Figure S6. Emission spectra for solutions of II (0.164 mM) and a solution of I (2.2 mM) with added II (0.164 mM) at high pH. The sharp peak at 580 nm is due to Rayleigh scatter arising from poor excitation of II at this excitation.

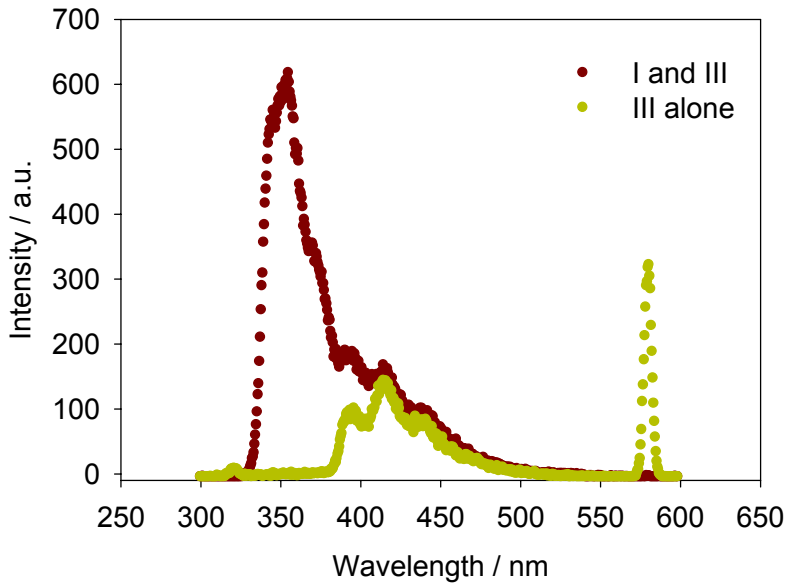

Figure S7. Emission spectra for solutions of III (0.060 mM) and a solution of I (2.2 mM) containing III (0.060 mM) at high pH. The sharp peak at 560 nm is due to Rayleigh scatter arising from poor excitation of III at this excitation.

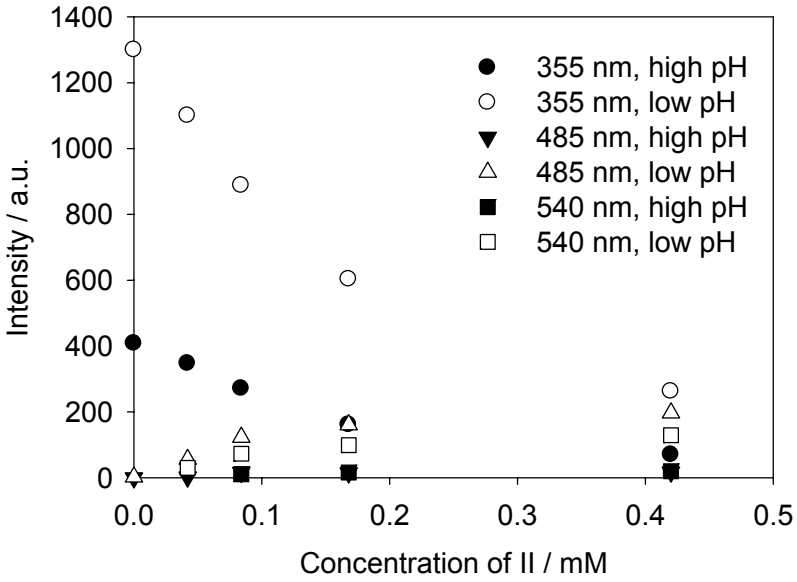

Figure S8. Variation in emission intensity at 355, 485 and 540 nm on addition of GdL (3.75 mg/mL) to a solution of I (2.2 mM) with added II. Without II, the intensity at 355 nm increases as gelation occurs. The increase is less apparent at low concentrations of II and decreases significantly at the higher concentrations of II. In all cases, the intensity at 485 nm

increases on gelation. Note, absolute intensities of the fluorescence at 355 nm for stock solutions of I are slightly variable between batches. We ascribe this to the aggregation at high pH being dependant on shear history on dissolution of I. However, all of the above data was recorded with the same stock solution of I and hence is comparable.

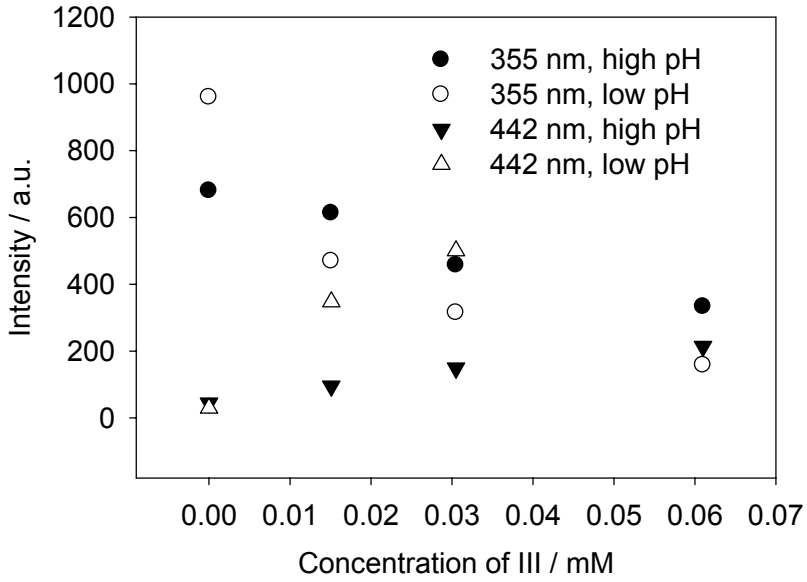

Figure S9. Variation in emission intensity at 355 and 442 nm on addition of GdL (3.75 mg/mL) to a solution of I (2.2 mM) with added III. Without III, the intensity at 355 nm increases as gelation occurs (the slight difference in absolute intensity is due to small differences in absolute concentration of dipeptide, which is self-quenched to some degree under these conditions). With added III, a decrease in intensity at 355 nm is observed at all concentrations of added III. In all cases, the intensity at 485 nm increases on gelation. Note, absolute intensities of the fluorescence at 355 nm for stock solutions of I are slightly variable between batches. We ascribe this to the aggregation at high pH being dependant on shear history on dissolution of I. However, all of the above data was recorded with the same stock solution of I and hence is comparable.

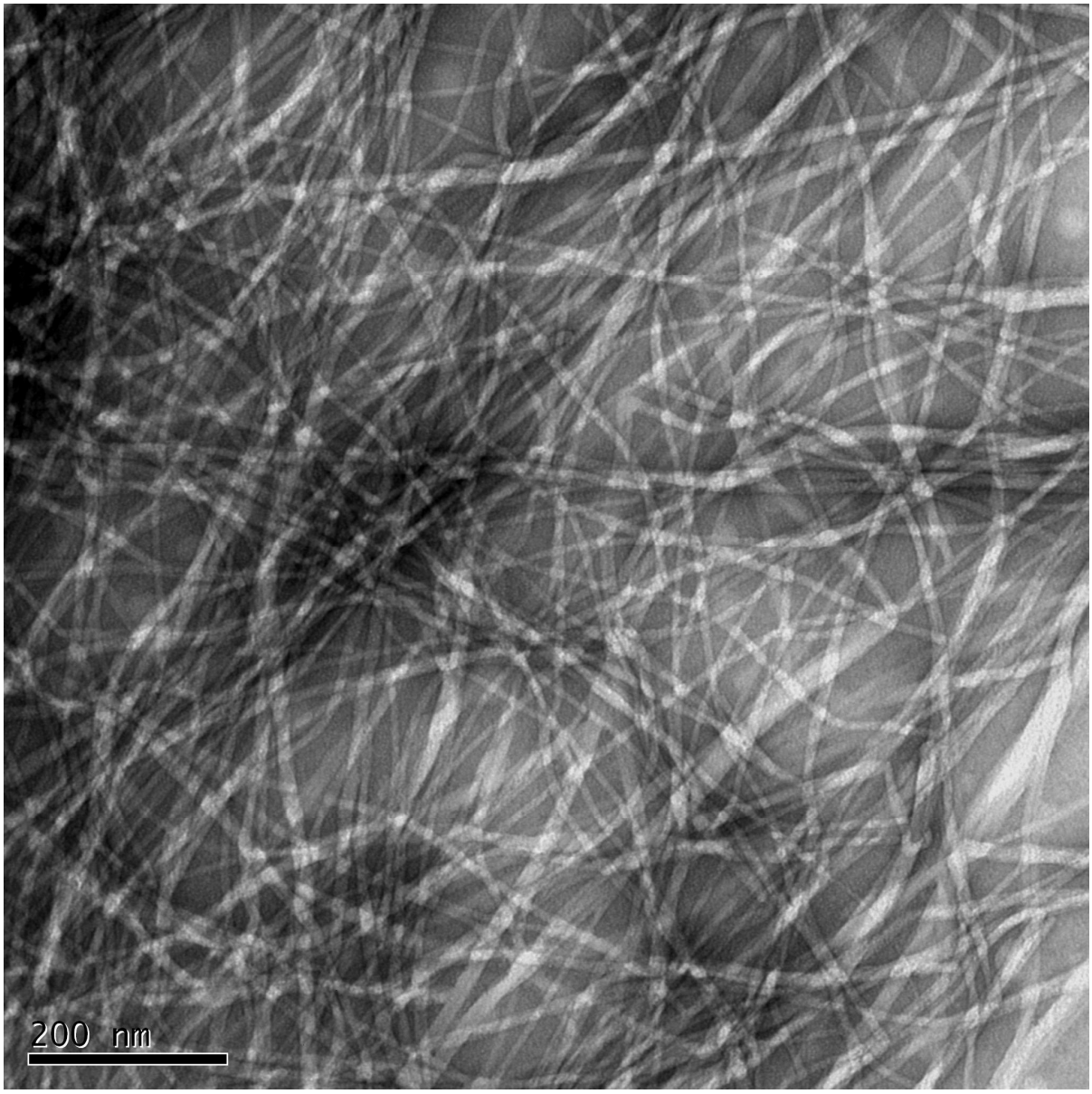

Figure S10. TEM image of hydrogel prepared from I as described above.

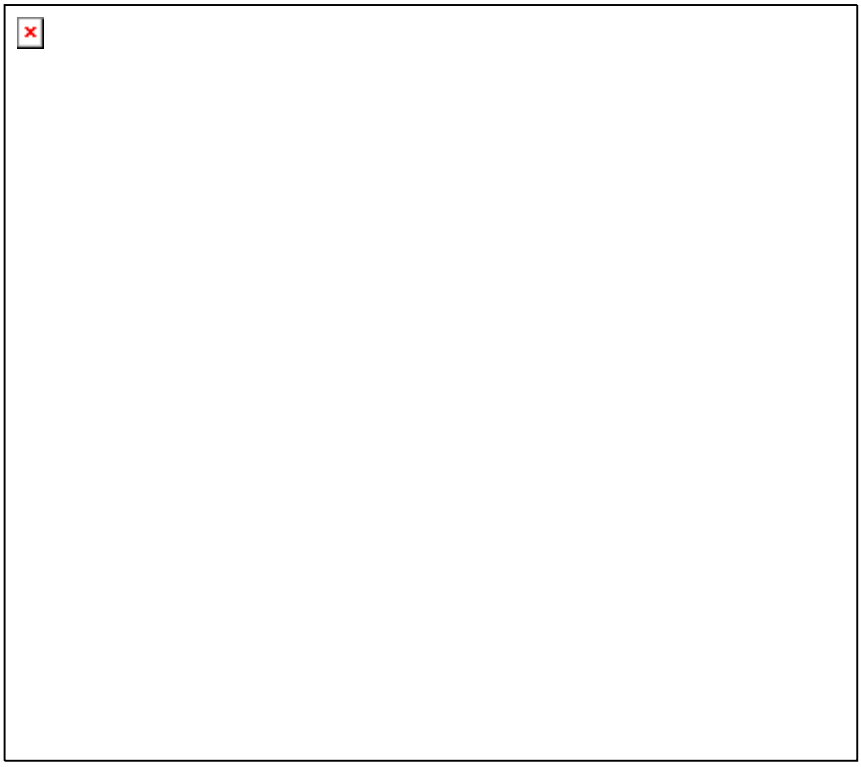

Figure S11. NMR of III (in d_6-DMSO) after precipitation from D_2O via addition of DCl (to a pD of approximately 2) to a solution at a concentration of 2.2 mM at pD 11. After standing at pD 2 for 2 hours, as much D_2O removed as possible from the precipitated solid by pipette and the solid dissolved in d_6-DMSO. The NMR demonstrates that III is stable at low pH. The peaks at 2.5 ppm and 3.8 ppm are solvent peaks.

References

1. W.A. Summers, J.Y. Lee and J.G. Burr, *J. Org. Chem.*, 1975, **40**, 1559-1561.

2. L. Chen, K. Morris, A. Laybourn, D. Elias, M.R. Hicks, A. Rodger, L. Serpell and D.J. Adams, *Langmuir*, 2010, **26**, 5232-5242.