

Self-Assembly and Hydrogelation Promoted by F₅-Phenylalanine

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ELECTRONIC SUPPORTING INFORMATION

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Materials. All amino acids except Fmoc-hFLeu and Cbz-F₅-Phe were purchased commercially from CSPS Pharmaceuticals, Inc. (San Diego, CA). Other solvents were purchased from Sigma-Aldrich and all materials were used without further purification. Fmoc-hFLeu was synthesized according to the methods of Marsh *et al.*¹ Cbz-F₅-Phe was synthesized according to the methods of Bosshard *et al.*² Water was filtered through a Barnstead nanopure water purification system prior to use in gel preparation.

Gel Preparation. Each monomer was dissolved in DMSO (210 mM, ~10 wt %). The stock solutions were then diluted with water to final concentrations of 4.2 mM (~0.2 wt % in 2:98 DMSO:H₂O, v/v). In the case of Fmoc-F₅-Phe, further dilution to a final concentration of 2.1 mM (~0.1 wt%) also resulted in hydrogelation. Following dilution each sample was briefly mixed by vortex (3–5 s) and was then allowed to rest at room temperature. Fmoc-F₅-Phe, initially an opaque suspension, became an optically transparent hydrogel in 5 min, while Fmoc-Tyr required 30–45 min before hydrogelation was observed. All other monomers precipitated from solution within 30 min.

Circular Dichroism (CD) Spectroscopy. CD spectra were recorded on an AVIV 202 Circular Dichroism spectrometer. CD spectra of the monomeric samples were recorded by dissolving monomer in MeOH. Gel samples were prepared by adding 100 μ L of 4.2 mM monomer in 2% MeOH:H₂O, immediately after dissolution of the monomer in water and before gelation had completed, into a 0.1 mm quartz cuvette. The gels were then allowed to set inside the cuvette and spectra were collected at 25 °C from 300 to 200 nm with a 1.0 nm step, 1.0 nm bandwidth, and 3 s collection time per step.

Scanning electron microscopy (SEM). Samples were prepared for imaging by spotting a 10 μ L aliquot of freshly prepared gel onto a carbon only TEM grid; the sample was allowed to stand for 1-2 min. The sample was then gently removed using capillary action and the grid was allowed to air dry. Images were taken on a Zeiss Supra 40VP FESEM operating with a 20 kV accelerating voltage.

Transmission electron microscopy (TEM). Images were taken with a Hitachi 7650 transmission electron microscope with an accelerating voltage of 80 kV. Samples were spotted directly onto 200 mesh carbon formvar copper grids and allowed to stand for 30-45 s. Excess solvent was carefully removed by capillary action (filter paper) and the grids were then immediately stained with uranyl acetate (20 μ L) for 30 seconds. Excess stain was removed by capillary action and the grids were allowed to air dry for 10–15 min.

Atomic force microscopy (AFM). Fresh gels were prepared and were then diluted 10 times with distilled water. Following dilution, 10 μ L was spotted onto freshly cleaved mica and allowed to stand 10–30 s. The substrate was then washed with 50 μ L of water and allowed to dry (~20 min). AFM images were acquired using silicon tips with a force constant of 2.8 N/m on a Digital Instruments Dimension Bioscope AFM with a Nanoscope IIIa controller operated in a tapping mode. Scans were taken with the following parameters: tapping frequency 75kHz, RMS amplitude of ~1.0 V before engage, integral and proportional gains 0.3–0.5, and 0.6–1.0 respectively, setpoint of 0.5–0.6V, and a scan rate of 1 Hz. All height analysis was completed

¹ J. T. Anderson, P. L. Toogood and E. N. G. Marsh, *Org. Lett.*, 2002, **4**, 4281–4283.

² H. R. Bosshard and A. Berger, *Helv. Chim. Acta*, 1973, **56**, 1838–1845.

using the cross section analysis featured in the Digital Instruments software. Reported heights are the average of at least 100 measurements on 100 different fibrils and the error is reported as the standard deviation about the mean.

Rheological Measurements. Measurements were conducted on a TA instruments AR-G2 rheometer operating in oscillatory mode, with a 20 mm parallel plate geometry equipped with a solvent trap filled with silicon oil in order to prevent evaporation. Samples were mixed and immediately applied to the stage (1.4 mm gap) and covered with the solvent trap in order to prevent sample evaporation. Following application of the sample to the stage a dynamic time sweep was immediately performed at 25 °C for 15–30 minutes with an angular frequency of 6 rad/s and 0.2% strain. A dynamic frequency sweep was taken immediately following the time sweep experiment over a range of frequencies from 0.1–100 rad s⁻¹ at 0.2% constant strain at 25 °C. Immediately following frequency sweeps, dynamic strain sweeps were performed at 0.1–100% strain with a constant frequency of 6 rad s⁻¹. A temperature sweep was performed at a constant frequency of 6 rad s⁻¹ with a constant strain of 0.2% over the temperature range of 25–85 °C, increasing by 1 °C per data point. All reported G' and G'' values represent an average of at least 3 independent samples.

XRD Measurements. Samples of gel were frozen and lyophilized to a dried powder and loaded into a quartz capillary tube. Two powder diffraction analyses were conducted. The first experiments were performed on a Philips MPD-Multipurpose Diffractometer with CuK α ($\lambda = 1.54$ Å) radiation. The instrument contained a 1° divergence slit, 15 mm mask, 1° anti-scatter slit and 0.45 mm receiving slit. The step size was 0.030 degrees for a count time of 10 s.

The second experiments were conducted using samples of gel from Fmoc-F₅-Phe that were frozen and lyophilized to a dried powder and loaded into a sample holder. Diffraction patterns were collected on a Bruker D8 Discover equipped with a HiStar area detector using CuK α ; ($\lambda = 1.54$ Å) radiation. Data was accumulated for 5 minutes. The resulting 2D diffraction images were integrated from 2.5 to 32.1 deg (2 theta) to produce standard intensity vs 2theta plots.

Solid State NMR. 19F magic-angle spinning (MAS) NMR data were obtained for unassembled Fmoc-F₅-Phe and lyophilized gel, both of which were loaded into 2.5 mm zirconia rotors. Spectra were collected at 11.7 T (470.2 MHz resonance frequency) using a Chemagnetics/Varian Infinity spectrometer and sample spinning in a commercial MAS NMR probe at 24 to 27 kHz. A 90° tip angle of 1 μ s and a recycle delay of 2 s were used to acquire 128 (lyophilized gel) and 1024 (unassembled Fmoc-F₅-Phe) transients. Data were processed with minimal apodization and plotted against an external shift reference of CFC1₃ at 0 ppm.

Molecular Modeling. Molecular modeling calculations were performed using Spartan (Wavefunction, Inc.). 4–8 Fmoc-F₅-Phe monomers were drawn in various starting conformations and the ensemble was minimized using the MMFFaq force field. The minimum structures obtained were all very similar to the structure shown in Figure 4.

UV Spectroscopy. The UV absorption of samples was obtained by preparing gels at a concentration of 4 mM in 2% MeOH/H₂O (v/v) in a 0.1 mm path length quartz cuvette. Spectra of the monomers were obtained by preparing a 4 mM solution of Fmoc-F₅-Phe in MeOH. Spectra were obtained using a Shimadzu UV-2401 PC UV-vis spectrophotometer. The spectra were recorded from 200 to 600 nm, and background was subtracted.

Fluorescence Spectroscopy. Fluorescence spectra were obtained using a Fluorolog-3 spectrofluorimeter. Samples of 4 mM Fmoc-F₅-Phe in methanol were prepared in a 0.1 mm path-path quartz cuvette. Samples of gels were prepared by adding 50 μ L of freshly mixed 4 mM Fmoc-F₅-Phe in 2% MeOH/H₂O into the cuvette and allowing hydrogelation to occur. Spectra were recorded from 295–500 nm, with the cutoff points dictated by the limits of the photomultiplier tube in the spectrofluorimeter.

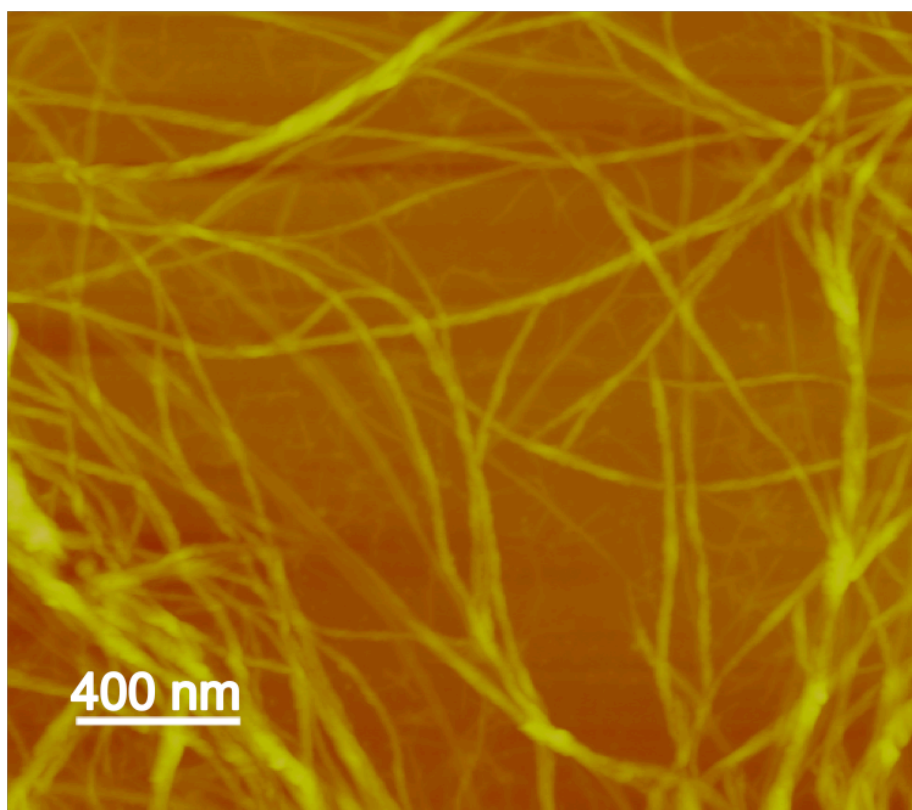


Figure S1. AFM image of fibrils derived from Fmoc-F₅-Phe hydrogel.

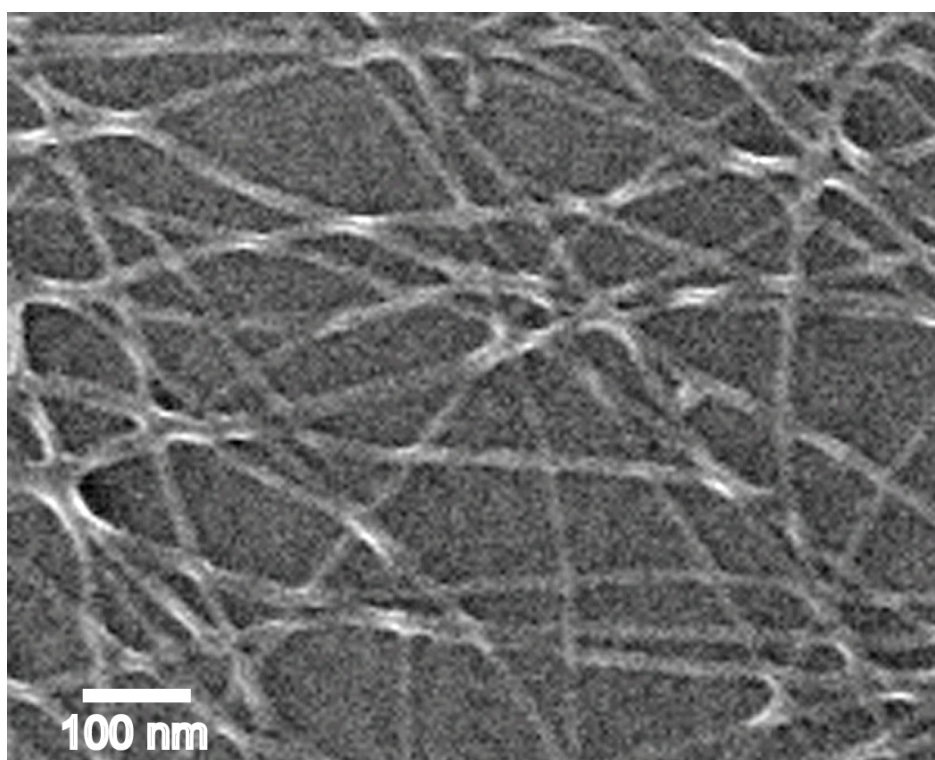


Figure S2. SEM image of fibrils derived from Fmoc-F₅-Phe hydrogel.

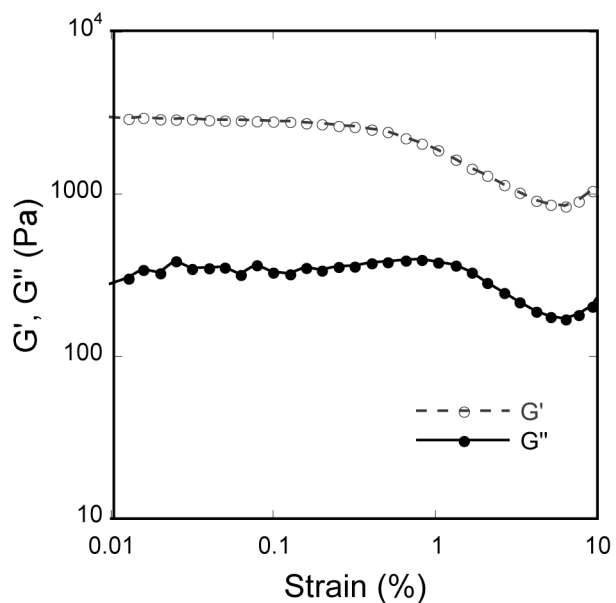


Figure S3. Rheological strain sweep of Fmoc-F₅-Phe hydrogel (4.2 mM, 2% DMSO/H₂O v/v).

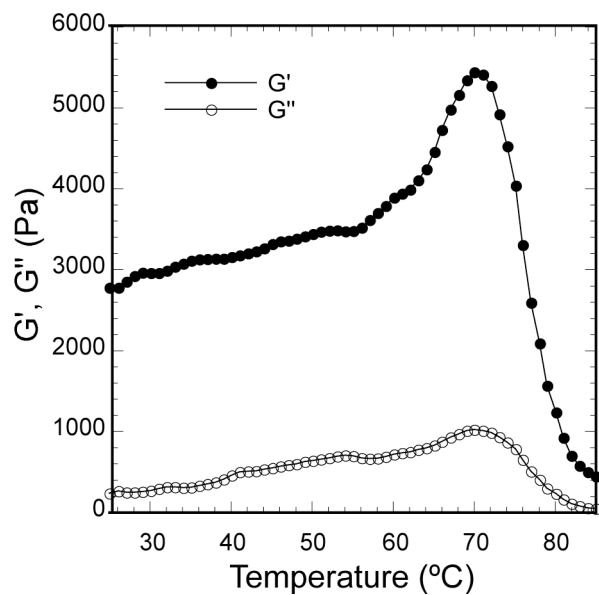


Figure S4. Rheological temperature sweep of Fmoc-F₅-Phe hydrogel (4.2 mM, 2% DMSO/H₂O v/v).

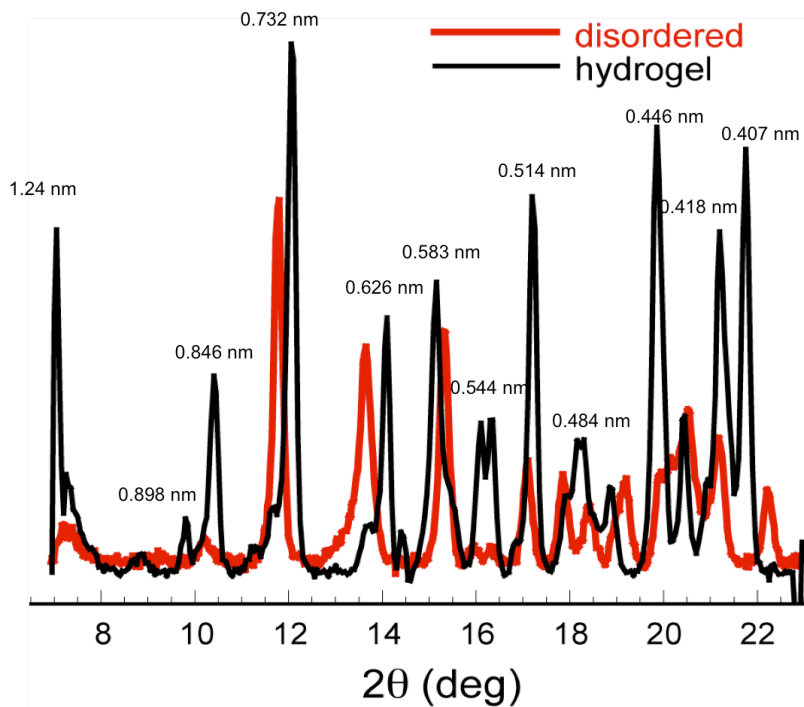


Figure S5. XRD diffraction (Philips MPD-Multipurpose Diffractometer) of lyophilized hydrogel and unassembled Fmoc-F₅-Phe powder. D-spacings for the hydrogel are labeled with the calculated value.

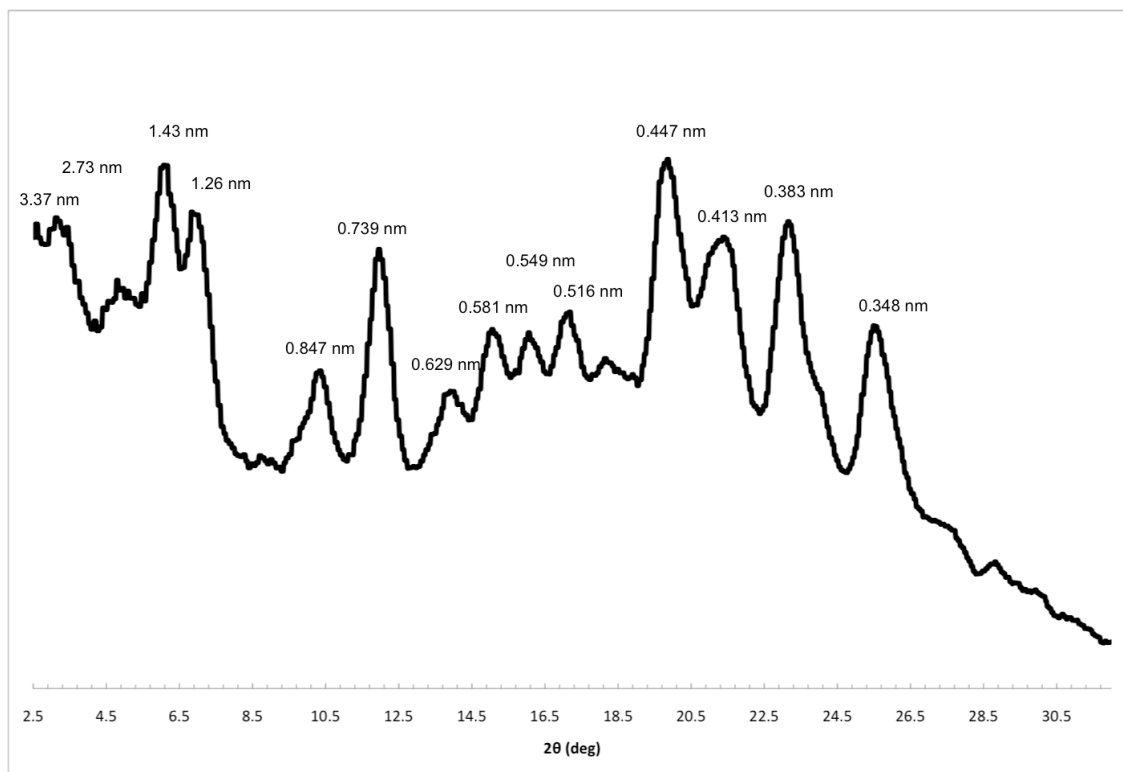


Figure S6. XRD diffraction (Bruker D8 Discover) of lyophilized Fmoc-F₅-Phe hydrogel.

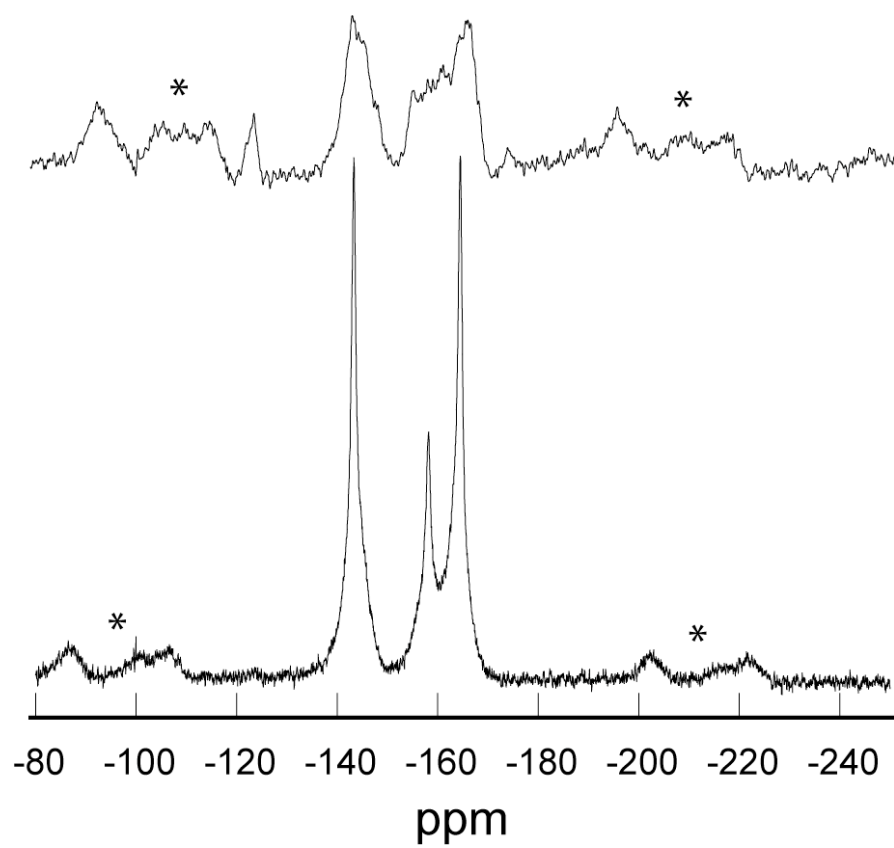


Figure S7. Solid-state ^{19}F -NMR of lyophilized gel (assembled) and unassembled Fmoc-F₅-Phe (* indicate spinning side-band regions).

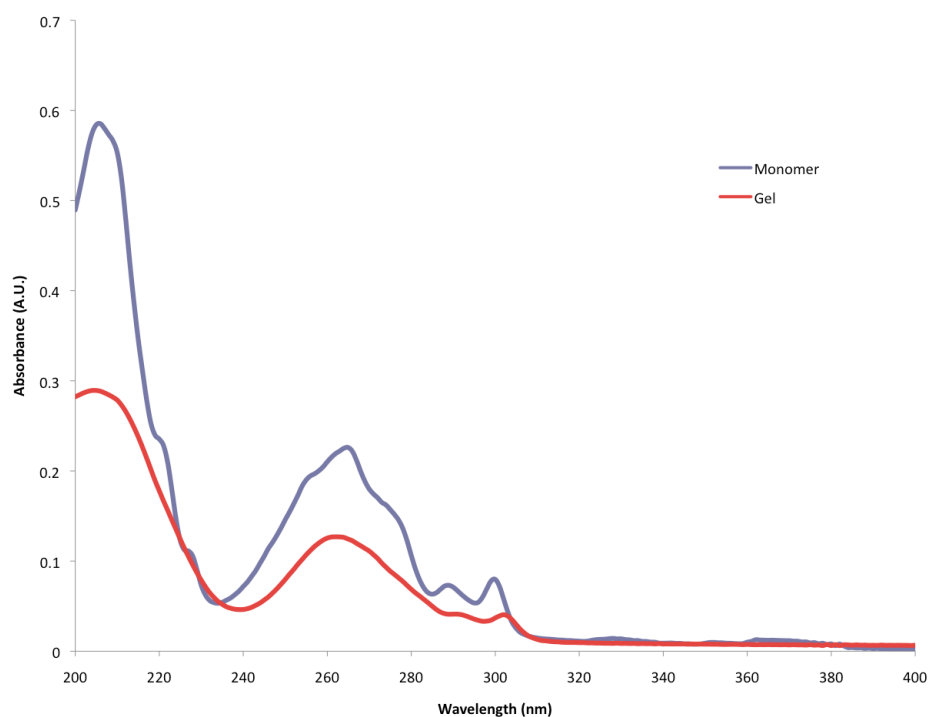


Figure S8. UV absorption spectra of monomeric Fmoc-F₅-Phe (4 mM, MeOH) and of the Fmoc-F₅-Phe hydrogel (4 mM, 2% MeOH/H₂O v/v).

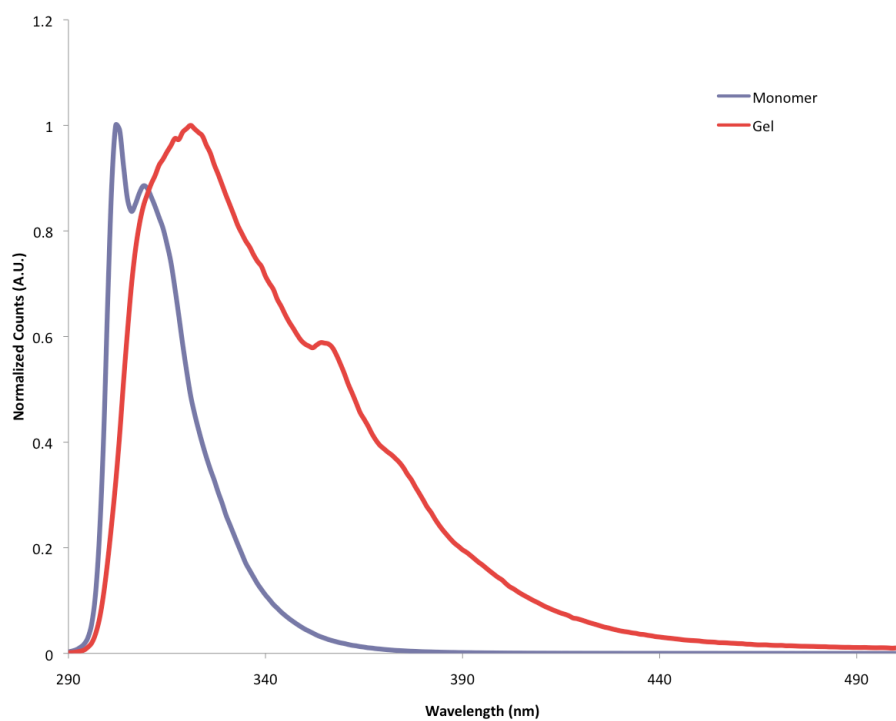


Figure S9. Fluorescence emission spectra of monomeric Fmoc-F₅-Phe (4 mM, MeOH) and of the Fmoc-F₅-Phe hydrogel (4 mM, 2% MeOH/H₂O v/v).