Supporting information for

A photo-crosslinking approach towards engineering small tyrosine-containing peptide hydrogels of enhanced mechanical stability

Yin Ding, Ying Li, Meng Qin, Yi Cao, and Wei Wang

HPLC analysis

High performance liquid chromatograph (HPLC) was undertaken in an Amersham Biosciences AKTA FPLC system including a P-900 pump, UV-900 detector, PH/C-900 and Frac-900 fraction collector with a GE SOURCE™ 5RPC ST 4.6/150 column. HPLC conditions: solvent A: 0.1% TFA in CH_3CN, solvent B: 0.1% TFA in H_2O, flow rate: 1 mL/min, gradient: 0 to 2.6 min, 1% A, 2.6 to 7.65 min, 1% to 5% A, 7.65 to 17.8 min, 5% to 99% A, 17.8 to 20.15 min, 99% to 100% A, 20.15 to 24 min, 100% to 99% A, 24 to 29 min, 99% A.

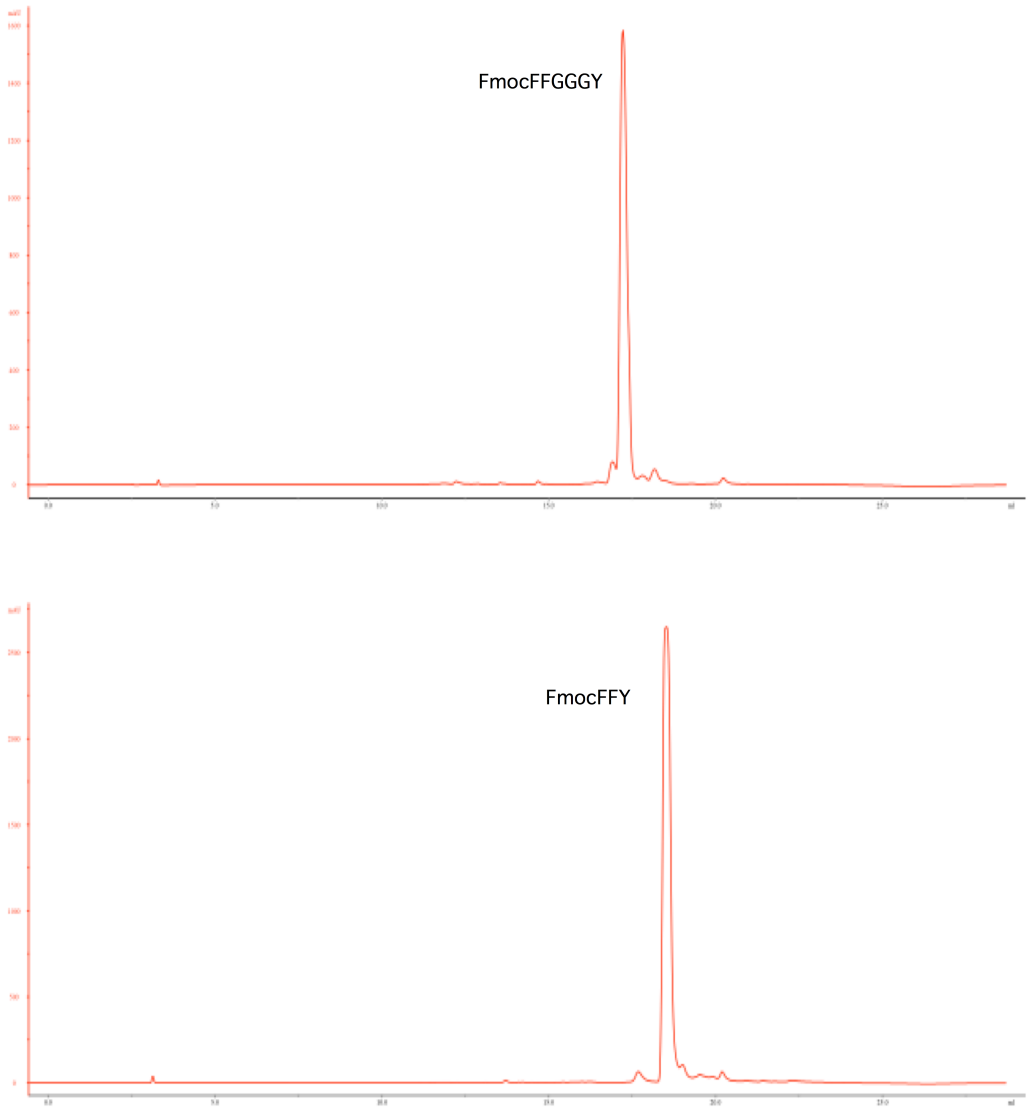


Figure S1 HPLC traces and of FmocFFGGGY and FmocFFY.

Gelation of FmocFFGGY and FmocFFY

To prepare 300 μL hydrogel (5 mg mL^{-1}), 15 μL DMSO solution of the peptide (100 mg mL^{-1}) was added to 285 μL 1× PBS (pH ~ 7). The mixture was then subject to vigorous shaking and then stored still at room temperature for at least 1 hr to allow the gelation to occur.

Ru^{2+} catalyzed photo-crosslinking for hydrogelation

To the 300 μL 5 mg mL^{-1} gel, $Ru(bpy)_3Cl_2$ in PBS (3.3 μL, 27.6 mM) and APS in PBS (15.6 μL , 0.178 M) was added respectively. Then the mixture was thoroughly mixed using vortexing and subsequently stored still for the gel to set. The yellow to orange gel like mixture was then irradiated with white light for a certain amount of time prior to the rheology experiments.

Rheology experiments

The gel was transferred to the rheometer plate with a spatula and the storage modulus (G') and loss modulus (G'') were measured with a Thermo Scientific Haake RheoStress 6000 in a frequency-sweep mode with geometry of 1° and 20 mm at 0.1% strain.

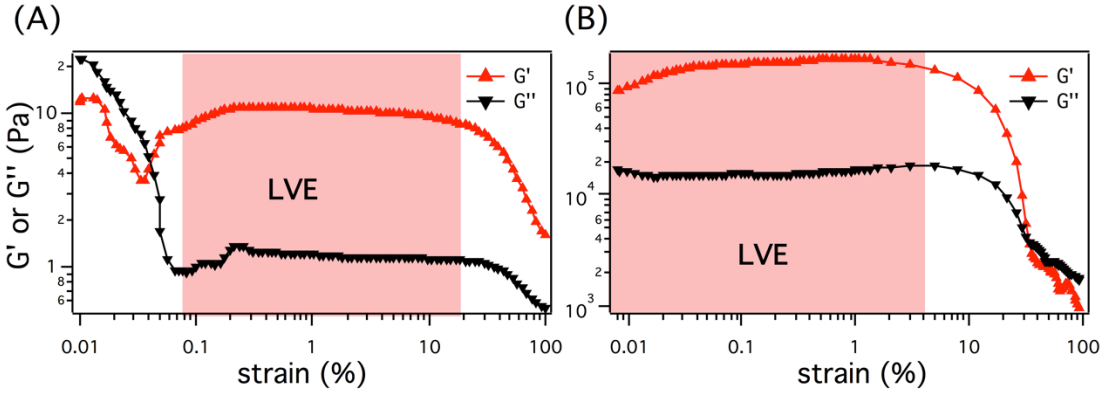


Figure S2 The strain sweep experiment for the FmocFFGGGY hydrogel before and after photo-irradiation. The strain-sweep experiment was undertaken at 1 Hz from strain amplitude from 0.01% to 100%: (A) FmocFFGGGY hydrogel, and (B) photo-crosslinked FmocFFGGGY hydrogel. The linear viscoelastic (LVE) region is highlighted in pink.

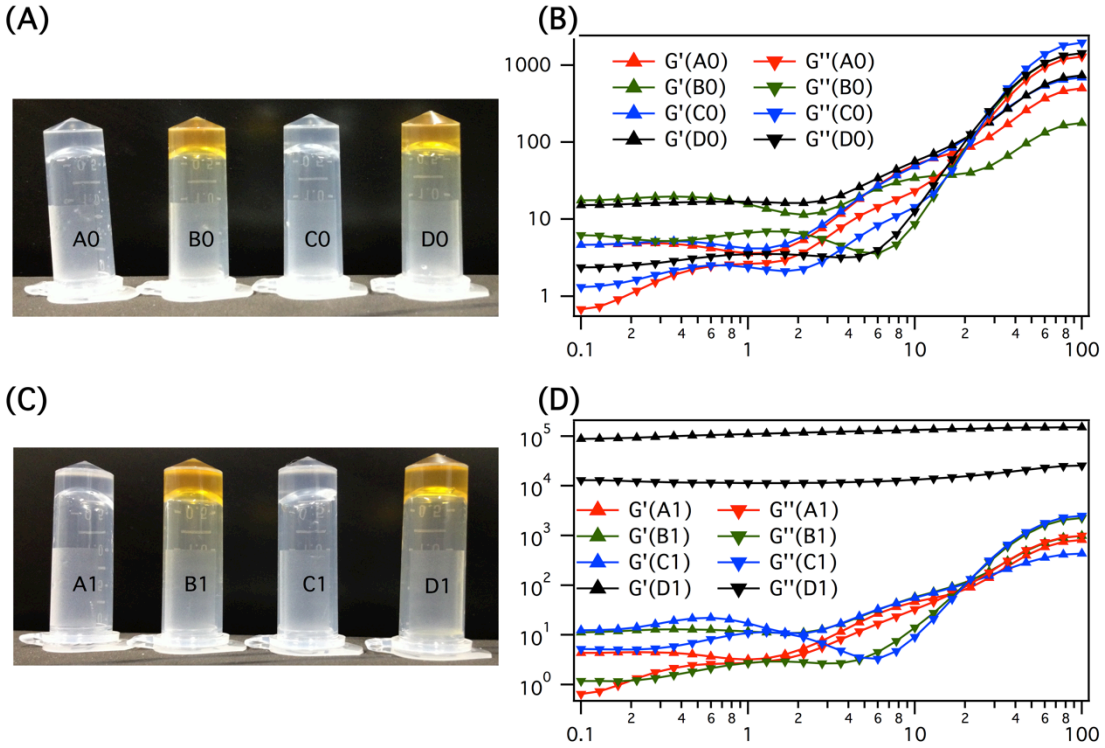


Figure S3 The role of each chemical adduct for crosslinking reaction in the dark (A and B) and under light irradiation (C and D). Left: the gel images (A), hydrogels kept in dark for 1 h at room temperature and the gel images (C), hydrogels subject to light irradiation for 2 min and then kept in dark for 1 h at room temperature; Right: the rheology experiments in the frequency-sweep mode (with 0.1% strain) after the gel images were taken: (B), that for the samples shown in (A), and (D), that for the samples shown in (C). For each tube: 5 mg mL^{-1} FmocFFGGGY (A0 and A1), 5 mg mL^{-1} FmocFFGGGY with 300 μM $Ru(bpy)_3Cl_2$ only (B0 and B1), 5 mg mL^{-1} FmocFFGGGY with 10.3 mM APS only (C0 and C1), and 5 mg mL^{-1} FmocFFGGGY with 286 μM $Ru(bpy)_3Cl_2$ and 10.2 mM APS (D0 and D1). It is worth noting that the uncrosslinked hydrogel (with or without the catalysts) is very fragile and prone to be destroyed by mild mechanical disturbance such that quickly inverting the tube could lead the hydrogel to deform into viscous liquid.

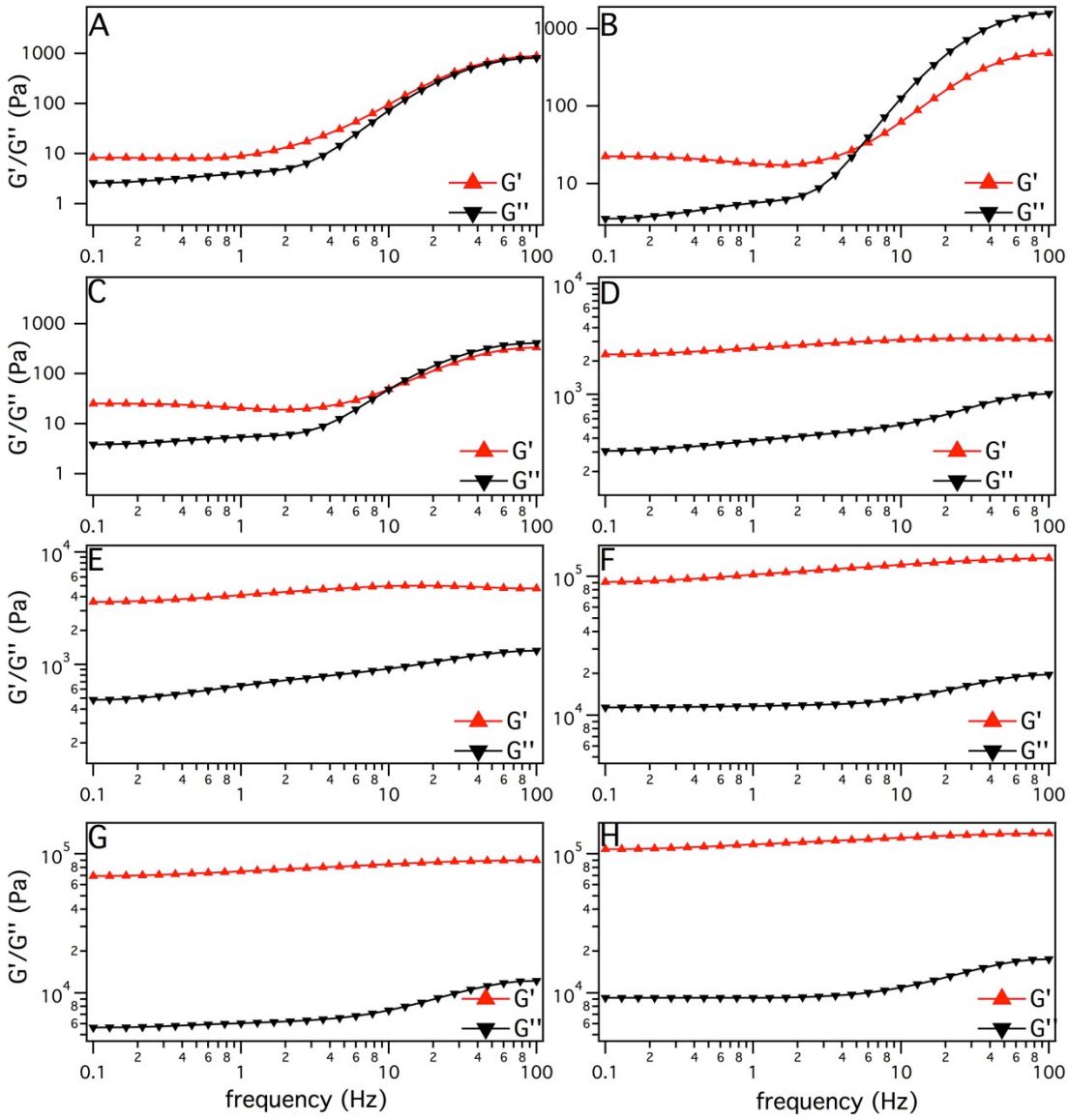


Figure S4 The rheology measurement of FmocFFGGGY before and after light irradiation. FmocFFGGGY hydrogel without light irradiation (A), B-H the FmocFFGGGY hydrogels after light irradiation for 0 s (B), 5 s (C), 10 s (D), 20 s (E), 30 s (F), 60 s (G) and 120 s (H).

Ru^{2+} catalyzed photo-crosslinking hydrogel investigated by fluorescent spectroscopy

After irradiation of the hydrogels, 20 μL hydrogel was diluted with 1.5 mL H_2O. Then 20 μL of the diluted solution was further diluted with 1.5 mL H_2O and the solution was used for the fluorescence measurement.

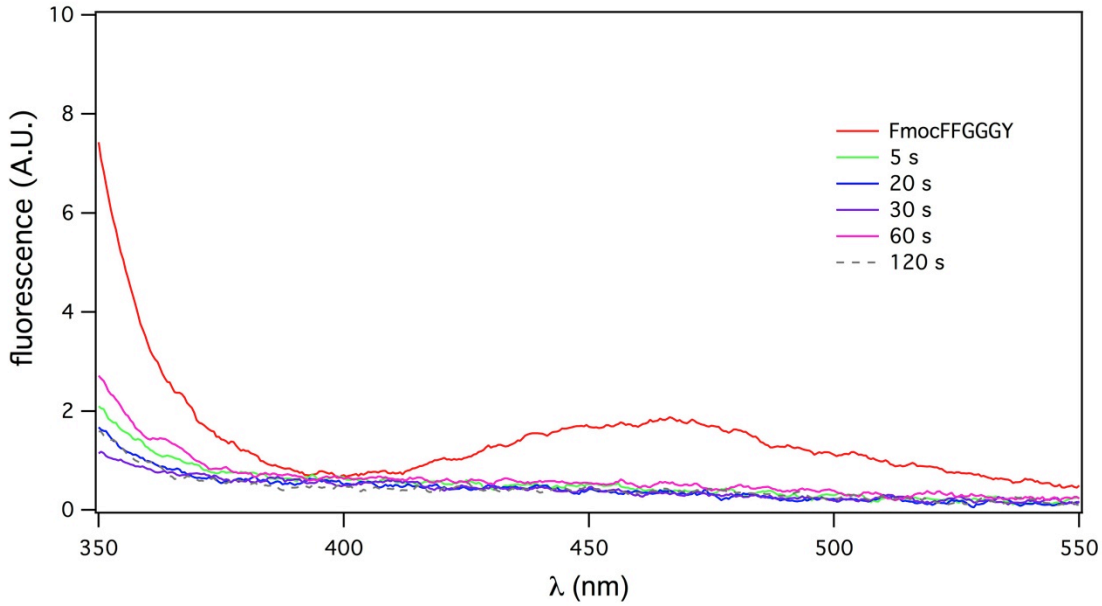


Figure S5 The fluorescence spectra of FmocFFGGGY hydrogels.

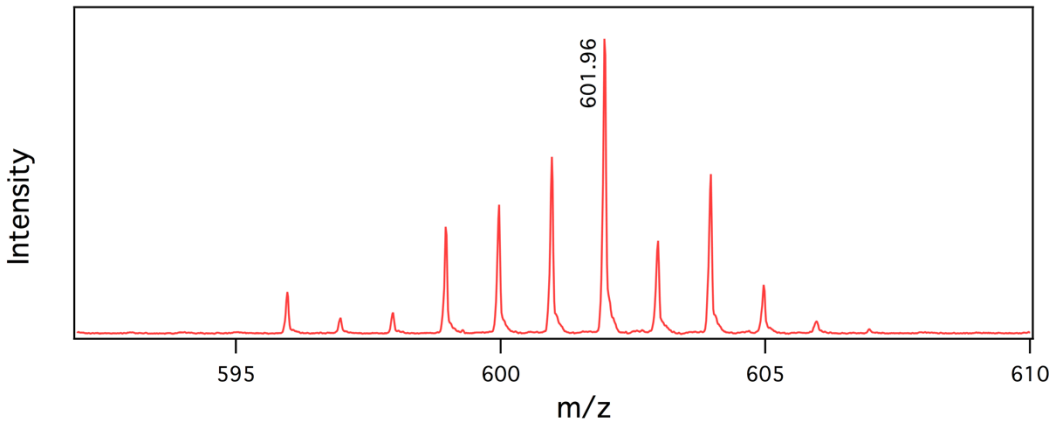


Figure S6 Mass spectrum for the detection of the dimerization of FmocFFGGGY upon photo-crosslinking. The photo-crosslinked FmocFFGGGY hydrogel was resuspended in 50% aqueous CH_3CN, vortexed thoroughly, and then loaded to the MALDI-TOF matrix α-cyano-4- hydroxycinnamic acid prior to the MALDI-TOF analysis.

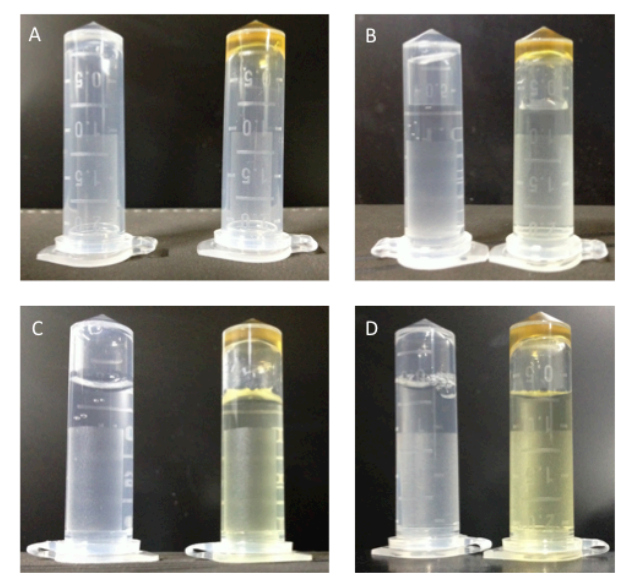


Figure S7 Crosslinking enhances the stability of FmocFFGGGY hydrogels in water. 200 μL of uncrosslinked (left) and crosslinked (right) hydrogels were immersed in deionized water (9 × volume of the hydrogel) for different time periods. The hydrogels resided in the bottom of the tubes and were inverted for picture only. Before the addition of water, (A); immediately after the addition of water, (B); after incubation in water for 3 days, (C); and 13 days, (D).

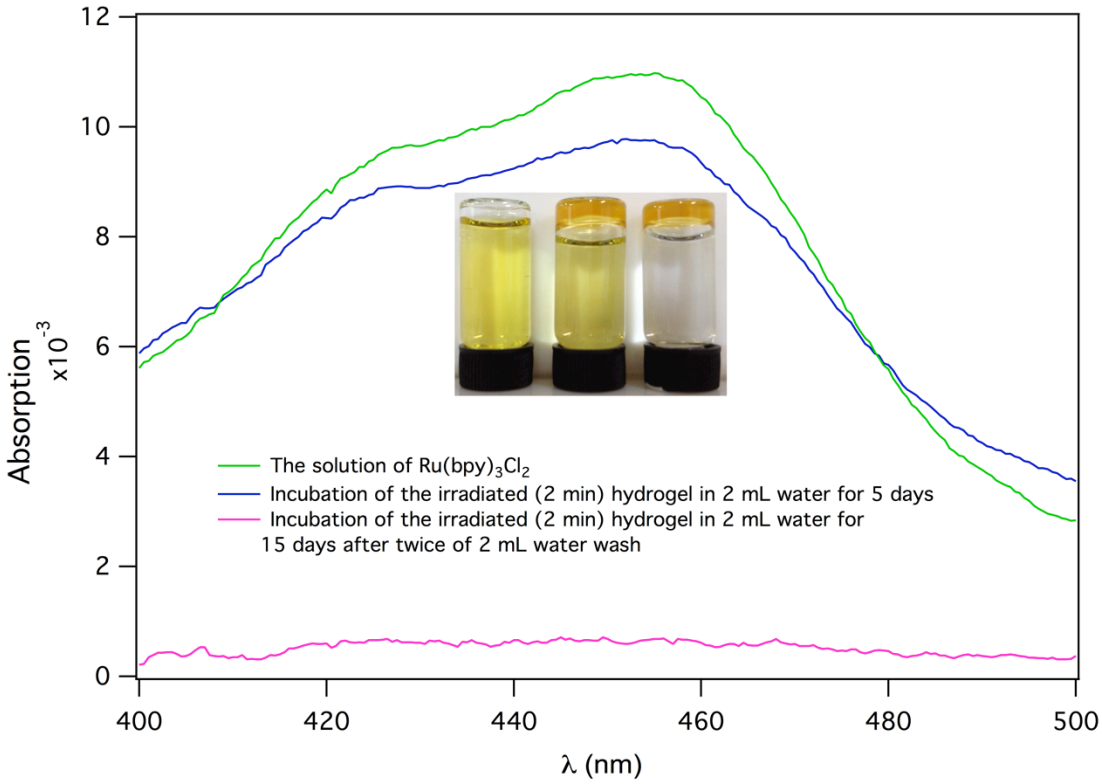


Figure S8 The UV-Vis spectra for the diffusion of $Ru(bpy)_3Cl_2$ into aqueous. For the UV-Vis spectroscopic measurement, all the samples (or supernatant) were diluted 50 times with water prior to the measurement. The absorption was recorded in the range of 500 nm to 400 nm for the characteristic absorption by $Ru(bpy)_3Cl_2$. The $Ru(bpy)_3Cl_2$ solution(left vial) was prepared by dissolving 3.3 μL 27.6 mM $Ru(bpy)_3Cl_2$ in 3.3 mL of water. The middle vial is the incubation of the photo-irradiated (2 min) hydrogel in 2 mL of water for 5 days and the right vial is the photo-irradiated (2 min) hydrogel that was washed twice with 2 mL water to remove any loosely attached peptides and $Ru(bpy)_3Cl_2$ and has been incubated in 2 mL of water for 15 days. Based on the calculation using the UV absorption, there was around 21% of the initial added $Ru(bpy)_3Cl_2$ trapped in the photo-crosslinked hydrogel.

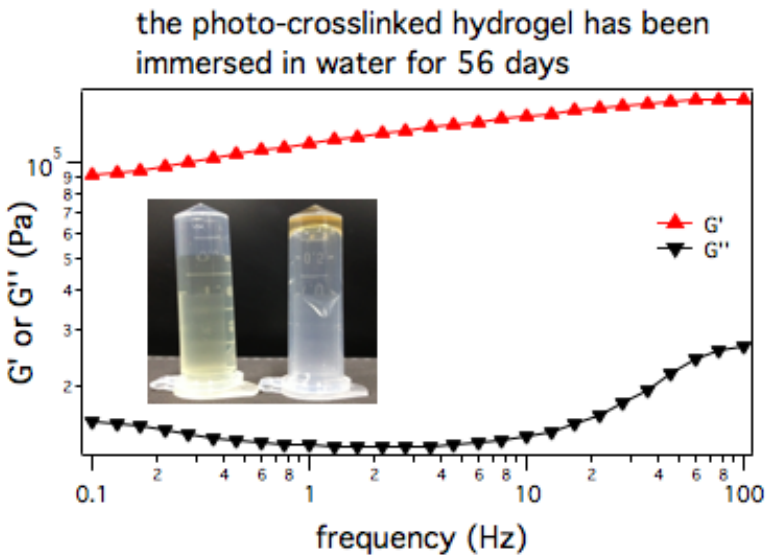
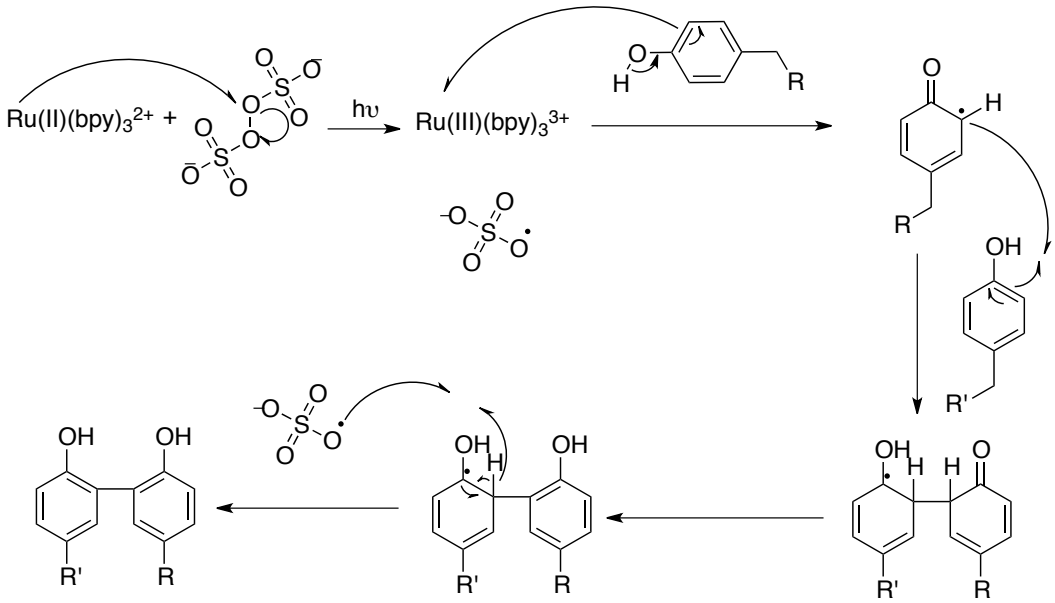


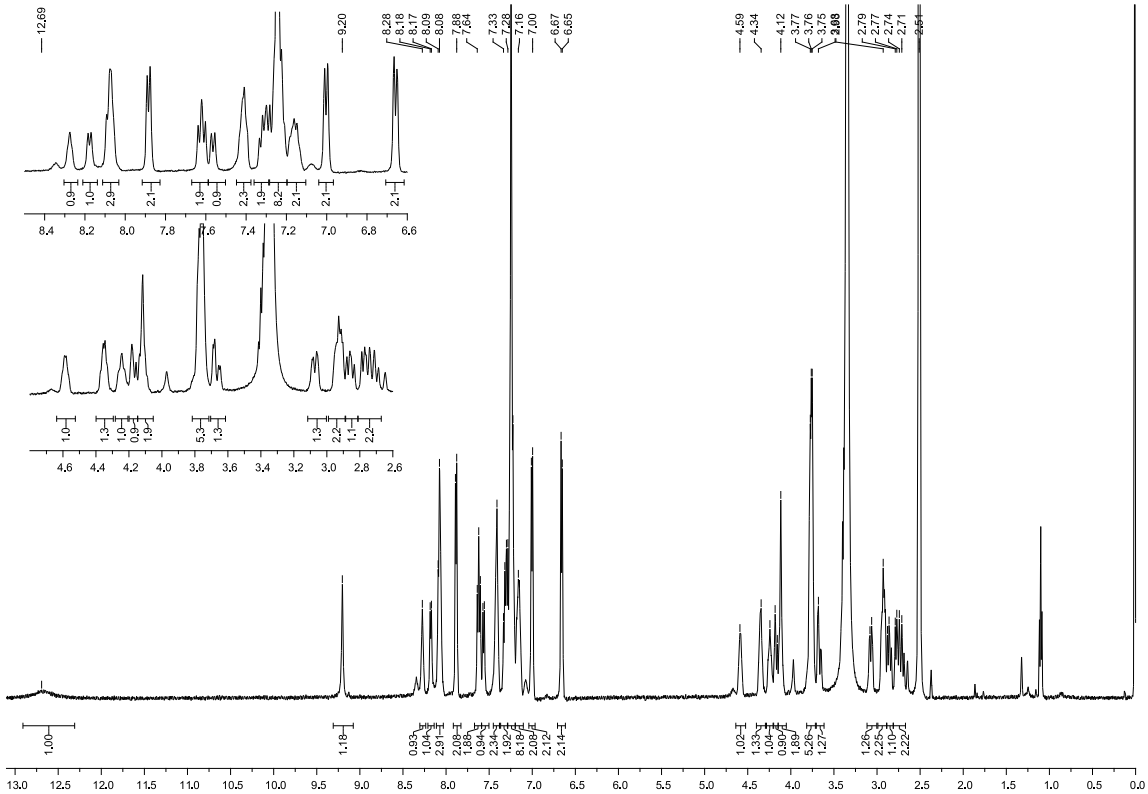
Figure S9 The rheology measurement of photo-crosslinked FmocFFGGGY hydrogel incubated in water after 56 days. The image inserted in the rheological data is showing the isolation of the supernatant (left) and the hydrogel (right) on day 56th. After this, the rheology measurement was carried out with a fixed strain of 0.1% in a frequency sweep mode.

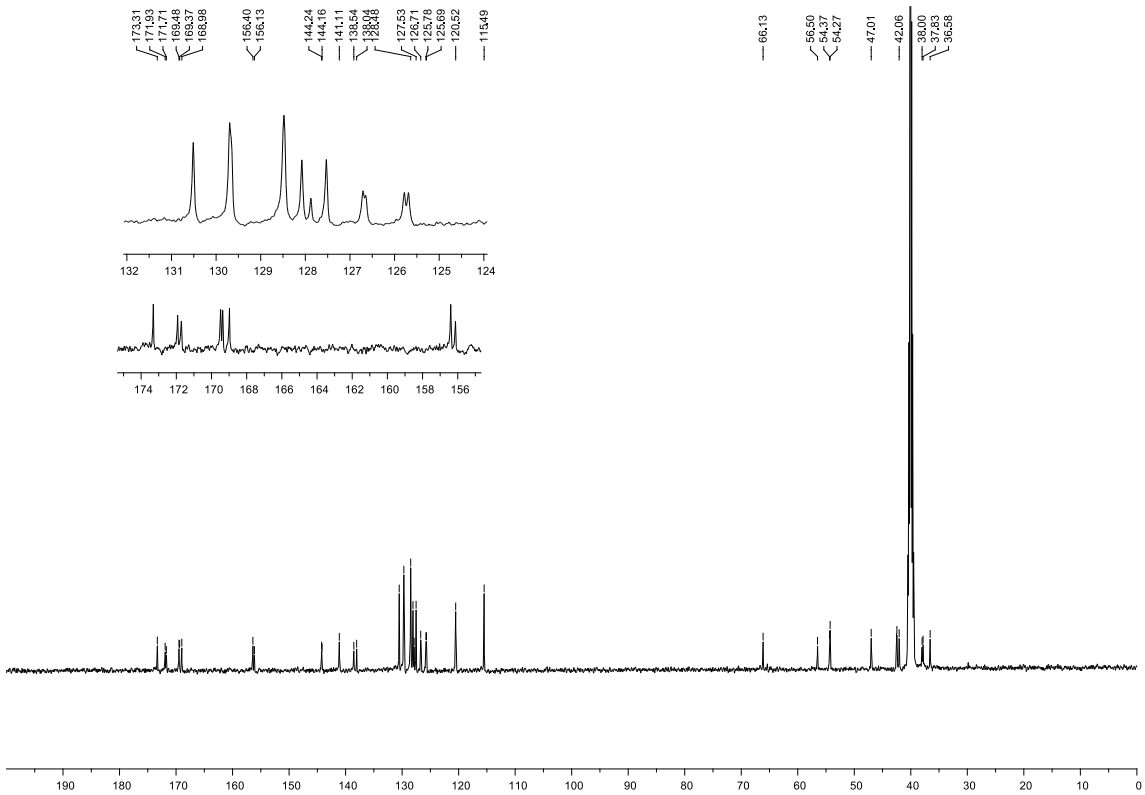


Scheme S1 The mechanism of Ru(II) catalyzed photo-crosslinking.[1]

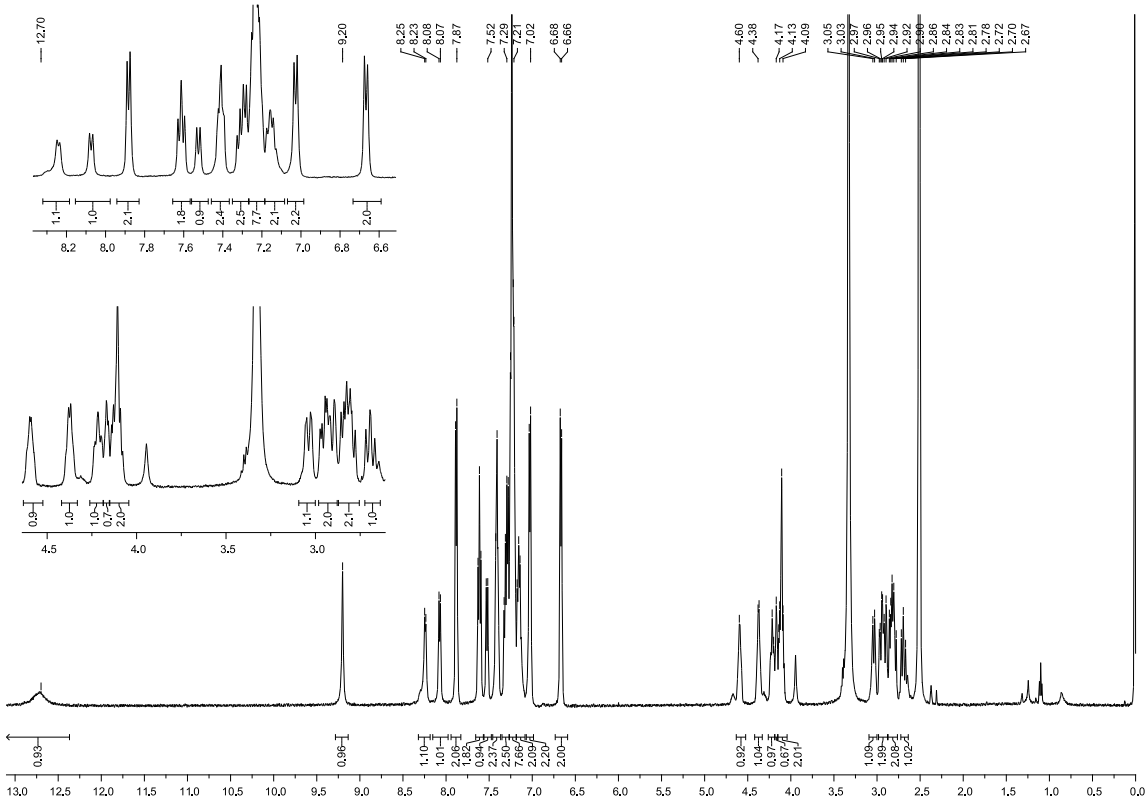
NMR spectra of the peptides

FmocFFGGGY

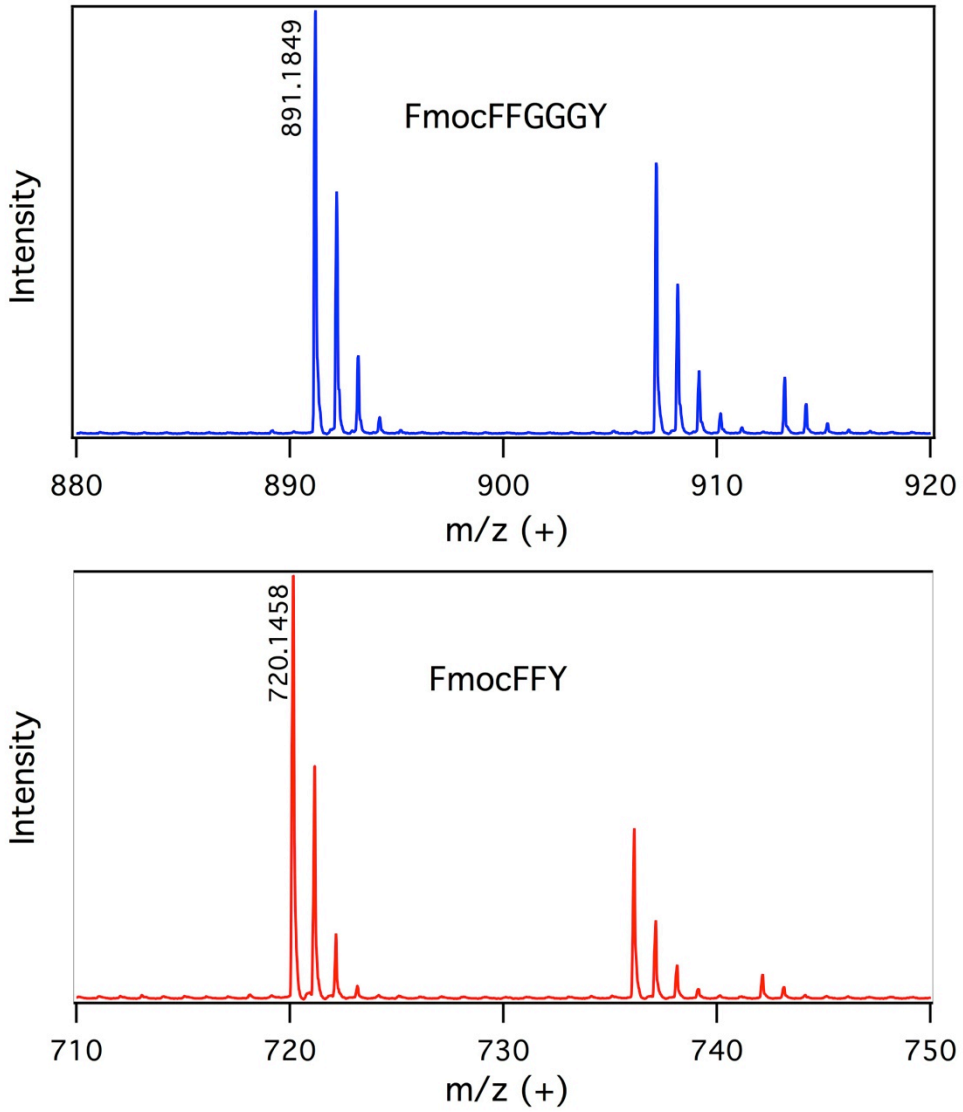




FmocFFY



Mass spectra of the peptides prepared vi Fmoc solid phase peptide synthesis.



(1) Fancy, D. A.; Kodadek, T., Chemistry for the analysis of protein-protein interactions: rapid and efficient cross-linking triggered by long wavelength light. *Proc. Natl. Acad. Sci. U. S. A.* **1999,** *96* (11), 6020-6024.