Supplementary Material (ESI) for Chemical Communications

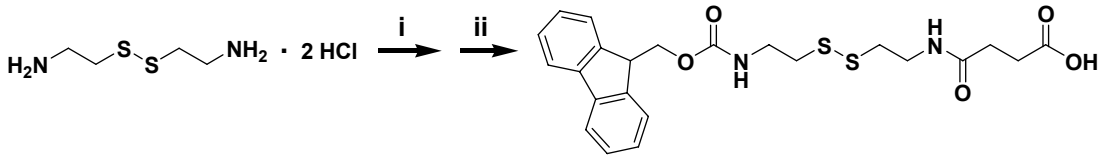

# Supporting Information

**Materials and general methods:**

**Chemicals:** Fmoc-OSu and other Fmoc-amino acid were obtained from GL Biochem (Shanghai); Chemical reagents and solvents were obtained from Alfa and used as received.

**General methods:** $^{1}$H NMR (Bruker ARX 400) was used to characterize the synthesized compounds. ESI-MS spectrometric analyses were performed at the Thermo Finnigan LCQ AD System. The ratio of **1**, **2**, and dimer of **1** was determined on the LCMS-20AD (Shimadzu). TEM was done on a Tecnai G2 F20 system, operating at 200 kV, TEM samples were prepared as following: a carbon-coated copper grid (from Zhongjingkeyi Technology Co. Ltd., Beijing, P. R. China) was vertically dipped into the hydrogels for 5 seconds, and then it was placed in a desicator overnight before the TEM measurement. Rheology test was done on an AR 2000ex (TA instrument) system, 40 mm parallel plates was used during the experiment at the gap of 500 μm.

**Peptide synthesis.** Peptide of Nap-GFFYE-CS-EERGD was prepared by standard Fmoc solid-phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding N-Fmoc protected amino acids with side chains properly protected by a tert-butyl group. The first amino acid (Fmoc-Asp(O$^{t}$Bu)-OH) was loaded on the resin at the C-terminal with the loading efficiency about 0.75 mmol/g. 20% piperidine in anhydrous N,N'-dimethylformamide (DMF) was used during the deprotection of Fmoc group. Then the next Fmoc-protected amino acid was coupled to the free amino group using O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU) as the coupling reagent. The growth of the peptide chain was according to the standard Fmoc SPPS protocol. In the last coupling step, 2-Naphalene acetic acid was used to cap the amine group of the peptide. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 minutes (5 ml per gram of resin), followed by five steps of washing using dichloromethane (DCM) for 2 min (5 ml per gram of resin). The peptide derivatives were cleaved from the resin by ice-cold reagent B and the mixture was stirred at room temperature, filtered, and poured into ice-cold diethylether, successively. The resulting precipitate was centrifuged for 10 min at 0-4 $^{0}$C at 10,000 rpm. Afterward the supernatant was decanted and the solid was dissolved in DMSO for HPLC purification.




**i: $NaHCO_3$, Succinic anhydride, dioxane/$H_2O$, 0 $^0$C**
**ii: Fmoc-OSu, DMF, 0 $^0$C**

***Scheme S-1.*** Synthetic route of Fmoc-cystamine succinic acid (Fmoc-CS) for solid phase peptide synthesis

**Synthesis and characterizations of Fmoc-cystamine succinic acid (Fmoc-CS):**

Cystamine dihydrochloride (2.25g, 10 mmol) and $NaHCO_3$ (2.52g, 30 mmol) was dissolved in 10 ml $H_2O$. And then 100 ml of Dioxane was added with stirring. After being cooled to 0 $^0$C in the ice bath for 10 minutes, 1.00g (10 mmol) of Succinic anhydride was added to the above mixture. The resulting reaction mixture was stirred overnight.

After the overnight reaction, 3.3 mL of DIPEA and 10 mL of DMF solution containing 3.373g (10 mmol) of Fmoc-OSu were successively added to the reaction mixture at 0 $^0$C in the ice bath. The reaction mixture was stirred for another 2 hours. A filtration through a filter paper was applied to remove the indiscerptible solid from the reaction mixture. The filtrate was then concentrated by the rotary evaporator. 200 mL of water was added to the resulting viscous liquid. And then the white precipitate was collected by filtration, washing with water, and dried in vacuum, successively. 2.04g (4.3 mmol) of the title product of Fmoc-CS was obtained by the flash column chromatography (1-10% Methanol in DCM) with a yield of 43%. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 7.85-7.88 (d, J=7.38 Hz, 2H); 7.65-7.68 (d, J=7.22 Hz, 2H); 7.39-7.42 (t, $J_1$=7.383 Hz, $J_2$=6.84 Hz, 2H); 7.34-7.28 (m, 2H); 4.29-4.32 (d, J=6.762 Hz, 2H); 4.19-4.23 (m, 1H); 2.78-3.34 (m, 4H); 2.73-2.78 (m, 4H); 2.40-2.44 (m, 2H); 2.28-2.33 (m, 2H). MS: calc. $M^+$ = 474.1, obsvd. $(M+1)^+$ = 475.5.

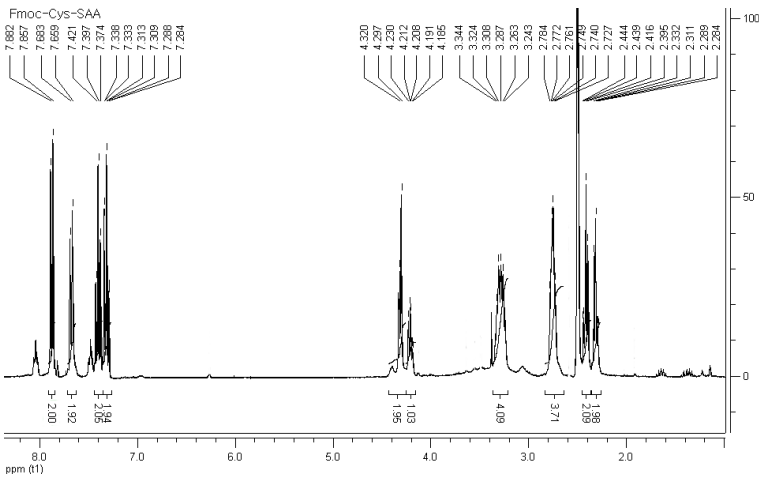



***Figure S-1***. $^1$H NMR of Fmoc-CS

**Characterization of 2:** $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 7.80-7.88 (m, 3H), 7.74 (s, 1H), 7.46-7.49 (m, 2H), 7.41 (d, 1H), 7.14-7.21 (m, 10H), 7.04 (d, 2H), 6.65 (d, 2H), 4.47-4.49 (m, 3H), 4.20-4.24 (m, 4H), 3.81-3.86 (d, 1H), 3.69-3.73 (m, 1H), 3.54-3.65 (m, 4H), 3.07-3.11 (m, 3H), 2.89-2.97 (m, 3H), 2.73-2.77 (m, 5H), 2.59-2.69 (m, 3H), 2.35 (s, 4H), 2.24-2.27 (m, 6H), 1.89-1.93 (m, 3H), 1.72-1.78 (m, 4H), 1.49-1.52 (m, 4H), 1.24 (m, 2H). MS: calc. $M^+$ = 1649.6, obsvd. $(M+1)^+$ = 1650.7, high resolution MS (HR-MS): calc. $M^+$ = 1650.62, obsvd. $(M+1)^+$ = 1651.6278

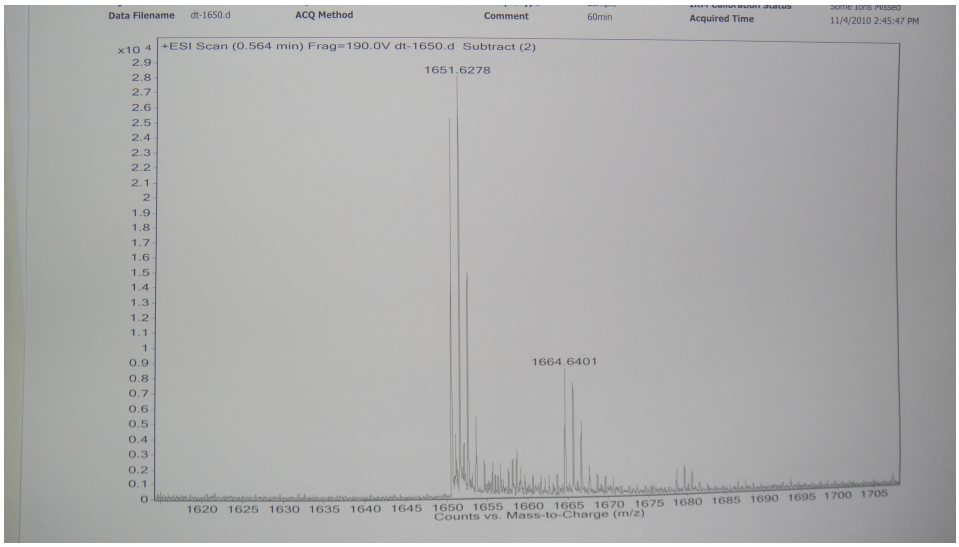



***Figure S-2***. HR-MS of Compound **2** (calc. $(M)^+$ = 1650.62, obsvd. $(M+1)^+$ = 1651.6278)

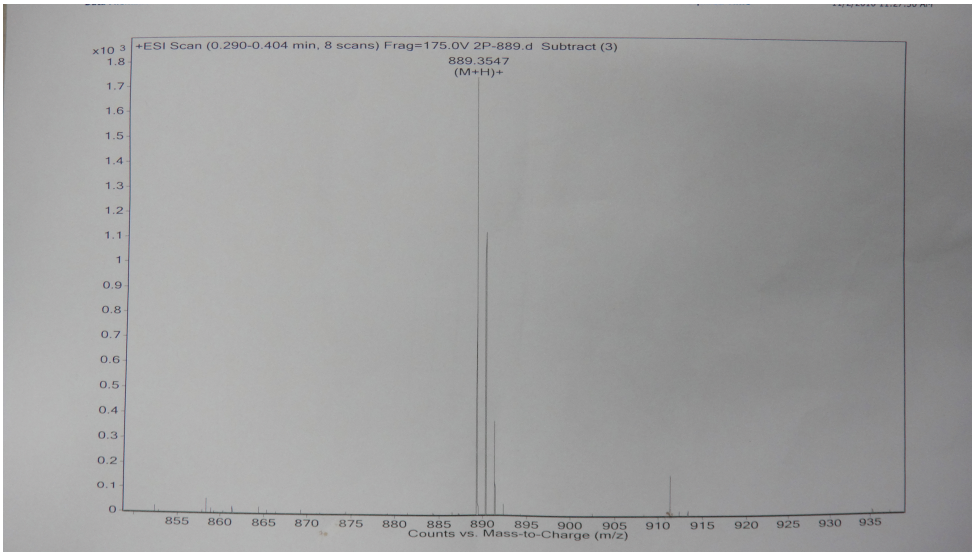



***Figure S-3***. HR-MS of Compound **1** (calc. $(M)^+$ = 888.35, obsvd. $(M+1)^+$ = 889.3547)

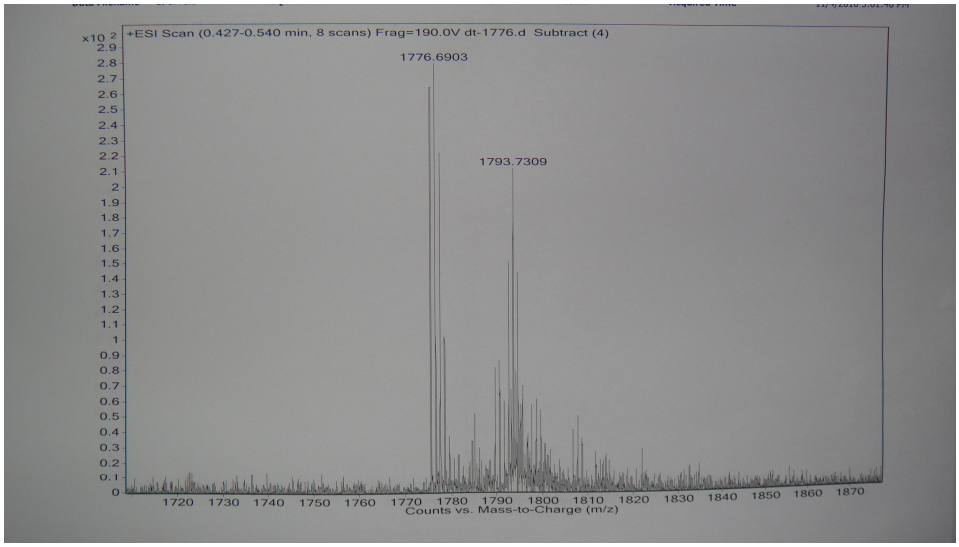



***Figure S-4***. HR-MS of dimer-**1** (calc. $(M)^+$ = 1775.69, obsvd. $(M+1)^+$ = 1776.6903)

**Formation of the hydrogel by TCEP or DTT in PBS buffer solutions:** 2.0 mg of **2** (1.21 μmol) were dissolved in 0.45 mL of PBS buffer solution containing 0.64 mg (5 equiv. to **2**) of $Na_2CO_3$ (3 equiv. of $Na_2CO_3$ were used to neutralize the carboxylic acids on **2** and the additional 2 equiv. of $Na_2CO_3$ were used to neutralize TCEP/DTT to make the final pH value of the resulting gel to about 7.4). And then 0.05 mL of PBS buffer solution containing 0.35 mg of TCEP (1.21 μmol, 1.0 equiv. to **2**)/0.19 mg of DTT (1.21 μmol, 1.0 equiv. to **2**) was added. Gels would form after being kept at room temperature (22-25 $^0$C) for about 10 and 8 minutes for TCEP and DTT, respectively.

**Formation of the hydrogel by GSH in PBS buffer solutions:** 2.0 mg of **2** (1.21 μmol) were dissolved in 0.45 mL of PBS buffer solution containing 0.90 mg (7 equiv. to **2**) of $Na_2CO_3$ (3 equiv. of $Na_2CO_3$ were used to neutralize the carboxylic acids on **2** and the additional 4 equiv. of $Na_2CO_3$ were used to neutralize GSH to make the final pH value to 7.4). And then 0.05 mL of PBS buffer solution containing 0.75 mg of GSH (2.42 μmol, 2 equiv. to **2**) was added. Gels would form after being kept at room temperature (22-25 $^0$C) for about 60 minutes.

**Formation of the hydrogel by TCEP/DTT/GSH in DMEM solutions:** The same procedure for the preparation of gels in PBS buffer solutions was used to form hydrogels in DMEM solution. The times needed for gelation post the addition of the reductants were shorter and they were about 3, 3, and 20 minutes when TCEP (1.0 equiv. to **2**), DTT (1.0 equiv. to **2**), and GSH (2 equiv. to **2**) were used, respectively.

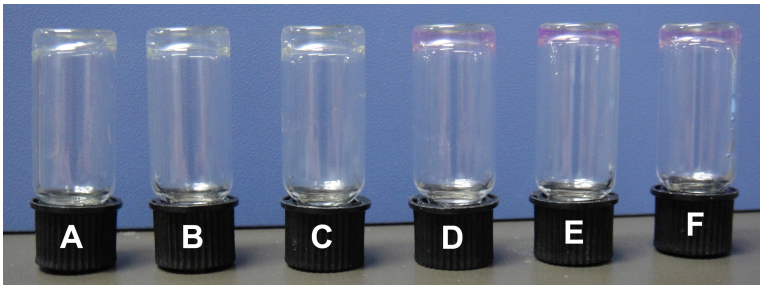



***Figure S-5***. Optical images of gels formed by treating the solution containing 0.4 wt% of **2** with A) 1.0 equiv. of TCEP in PBS buffer solution, B) 1.0 equiv. of DTT in PBS buffer solution, C) 2 equiv. of GSH in PBS buffer solution, D) 1.0 equiv. of TCEP in DMEM solution, E) 1.0 equiv. of DTT in DMEM solution, and F) 2.0 equiv. of GSH in DMEM solution

**Determination of the percentage of 2, 1, and dimer of 1 in hydrogels:** The 25 μL of the hydrogels at different time points was dissolved in 100 μL of DMSO solutions. And then a 20 μL of the resulting solution was used to run the LC-MS on a LCMS-20AD (Shimadzu) system. The areas of different peaks at 272 nm were used to calculate the percentage of the compounds in the hydrogels. The experiment was repeated for 3 times.

***Table S-1.*** Analysis of molar percentage of **1**, **2**, and dimer of **1** by LC-MS in gels formed by 1.0 equiv. of TCEP in PBS buffer solutions at different time points

| Time (h) | 1 | 2 | Dimer of 1 |
|---|---|---|---|
| Gelling point (10 minutes) | >99.0% | <0.5% | <0.5% |
| 0.5 | >98.8% | <0.5% | 0.7% |
| 1 | 98.0% | 0.4% | 1.6% |
| 2 | 96.1% | 0.5% | 3.4% |
| 6 | 95.8% | 0.5% | 3.7% |
| 12 | 94.4% | 1.1% | 4.5% |
| 24 | 91.0% | 1.5% | 7.5% |
| 48 | 62.4% | 2.2% | 35.4% |
| 72 | 42.8% | 2.9% | 54.3% |
| 120 | 1.0% | 3.5% | 95.5% |
| 168 | 0.5% | 3.7% | 95.8% |

**Note:** The total area of peaks of **1**, **2**, and dimer of **1** was set to be 100%. The gelation time was 10 minutes after the addition of 1.0 equiv. of TCEP to a PBS solution containing 0.4 wt% of **2**.

***Table S-2.*** Analysis of molar percentage of **1**, **2**, and dimer of **1** by LC-MS in gels formed by 1.0 equiv. of TCEP in DMEM solutions at different time points

| Time (h) | 1 | 2 | Dimer of 1 |
|---|---|---|---|
| Gelling point (3 minutes) | >93.3% | 6.2% | <0.5% |
| 0.5 | >96.8% | 2.7% | <0.5% |
| 1 | 96.7% | 2.0% | 1.3% |
| 2 | 94.3% | 0.8% | 4.9% |
| 6 | 90.0% | 0.6% | 9.4% |
| 12 | 83.2% | 1.7% | 15.1% |
| 24 | 61.5% | 1.9% | 36.6% |
| 48 | 42.3% | 2.1% | 55.6% |
| 96 | 18.5% | 5.1% | 76.4% |
| 120 | 4.2% | 7.7% | 88.1% |
| 168 | 0.7% | 9.8% | 88.6% |

**Note:** The total area of peaks of **1**, **2**, and dimer of **1** was set to be 100%. The gelation time was ~3 minutes after the addition of 1.0 equiv. of TCEP to a DMEM solution containing 0.4 wt% of **2**.

***Table S-3.*** Analysis of molar percentage of **1**, **2**, and dimer of **1** by LC-MS in gels formed by 2.0 equiv. of TCEP in PBS buffer solutions at different time points

| Time (h) | 1 | 2 | Dimer of 1 |
|---|---|---|---|
| Gelling point (6 minutes) | > 99% | < 0.5% | < 0.5% |
| 0.5 | > 99% | < 0.5% | < 0.5% |
| 1 | > 99% | < 0.5% | < 0.5% |
| 2 | > 99% | < 0.5% | < 0.5% |
| 6 | > 99% | < 0.5% | < 0.5% |
| 12 | > 99% | < 0.5% | < 0.5% |
| 24 | > 99% | < 0.5% | < 0.5% |
| 48 | > 99% | < 0.5% | < 0.5% |
| 72 | > 99% | < 0.5% | < 0.5% |
| 120 | > 99% | < 0.5% | < 0.5% |
| 168 | > 99% | < 0.5% | < 0.5% |

**Note:** The total area of peaks of **1**, **2**, and dimer of **1** was set to be 100%. The gelation time was 6 minutes after the addition of 2.0 equiv. of TCEP to a PBS solution containing 0.4 wt% of **2**.

***Table S-4.*** Analysis of molar percentage of **1**, **2**, and dimer of **1** by LC-MS in gels formed by 2.0 equiv. of GSH in DMEM solutions at different time points

| Time (h) | 1 | 2 | Dimer of 1 |
|---|---|---|---|
| Gelling point (3 minutes) | 27.5% | 27.6% | 44.9% |
| 0.5 | 16.8% | 27.5% | 55.7% |
| 1 | 15.5% | 25.0% | 59.5% |
| 2 | 7.4% | 24.9% | 67.7% |
| 6 | 0.8% | 23.5% | 75.7% |
| 12 | 0.6% | 20.5% | 78.9% |
| 24 | 0.9% | 20.2% | 78.9% |
| 48 | 0.3% | 17.1% | 82.6% |
| 72 | 0.3% | 17.1% | 82.6% |
| 120 | 0.3% | 17.1% | 82.6% |
| 168 | 0.3% | 17.1% | 82.6% |

**Note:** The total area of peaks of **1**, **2**, and dimer of **1** was set to be 100%. The gelation time was ~10 minutes after the addition of 2.0 equiv. of GSH to a DMEM solution containing 0.4 wt% of **2**.

**Rheology:** Rheology test was done on an AR 2000ex (TA instrument) system, 40 mm parallel plates was used during the experiment at the gap of 500 □m. For the dynamic time sweep, the solution of **2** after the addition of 1 equiv. of TCEP was directly transferred to the rheometer and it was conducted at the frequency of 2 rad/s and the strain of 2%. The gels were also characterized by the mode of dynamic frequency sweep in the region of 0.1-100 rad/s at the strain of 2%. The recovery property of the gels was probed as following: the large amplitude of stain (50%) at the frequency of 2 rad/s was firstly applied to the gels for 600 seconds. And then the recovery property of the gels was measured right after the removal of the external large amplitude of force (50% of strain) at the strain of 2% and the frequency of 2 rad/s for 3,600 seconds.

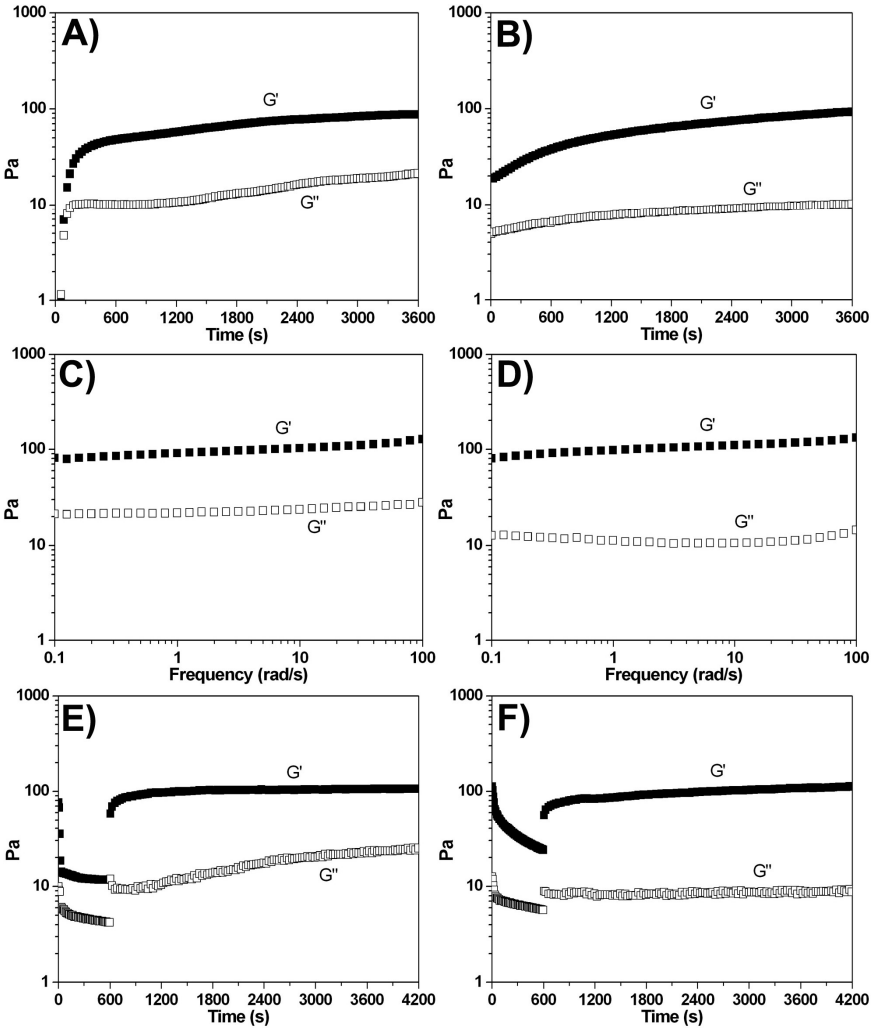



***Figure S-6***. Dynamic time sweep of solutions containing 0.4 wt% of **2** with 1 equiv. of TCEP at the strain of 2% and the frequency of 2 rad/s A) in the PBS buffer solution and B) in the DMEM solution, dynamic frequency of gels formed by treating solutions containing 0.4 wt% of **2** with 1 equiv. of TCEP C) in the PBS buffer solution and D) in the DMEM solution, and the recovery property of the resulting hydrogel E) in the PBS buffer solution and F) in the DMEM solution (the gel was firstly subjected to a large amplitude of strain of 50% at the frequency of 2 rad/s for 600 seconds and then its recovery was probed at the strain of 2% and the frequency of 2 rad/s for 3,600 seconds)

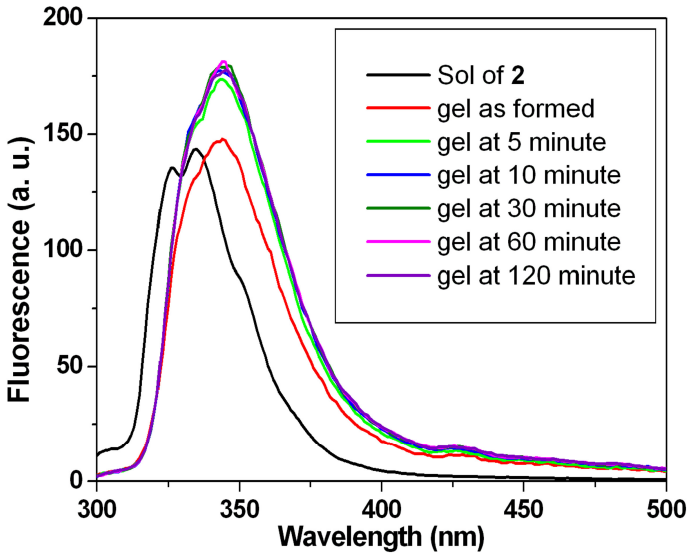



***Figure S-7***. Emission spectra of PBS buffer solution of **2** and gels formed by treating PBS buffer solution of **2** with 1 equiv. of TCEP at different time scale ( $\lambda_{exc}$ = 272 nm)

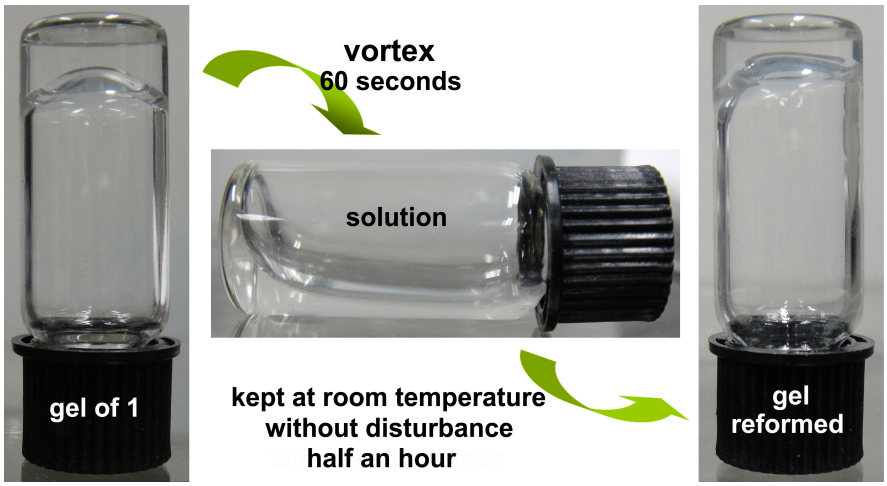



***Figure S-8***. Optical images to show that the gel as formed is a thixotropic one: left) gel formed by treating solution of **2** (0.4 wt%) with 1 equiv. of TCEP in the PBS buffer solution after 1 hour, middle) solution formed from the gel in left) by vortex for 60 seconds, and right) the gel reformed by keeping the solution in middle) at room temperature without disturbance for half an hour

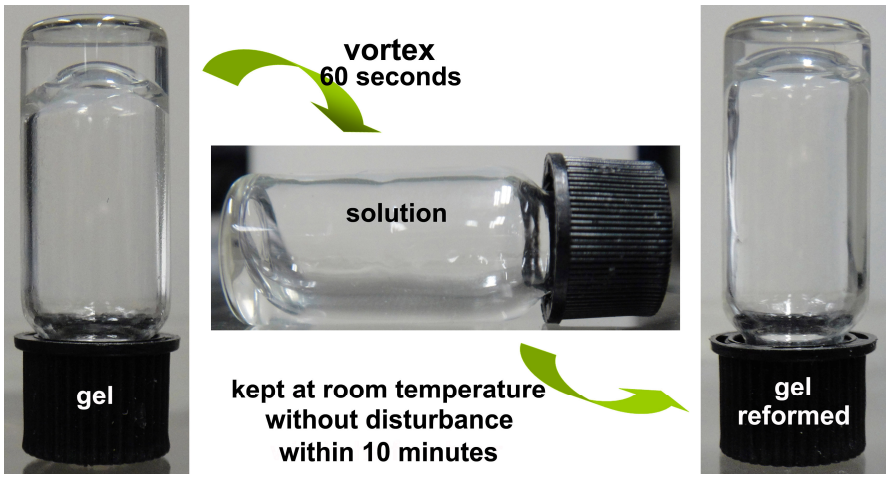



***Figure S-9***. Optical images to show that the gel formed after 48 hours is also a thixotropic one: left) gel formed by treating solution of **2** (0.4 wt%) with 1 equiv. of TCEP in the PBS buffer solution after 48 hours, middle) solution formed from gel in left) by vortex for 60 seconds, and right) the gel reformed by keeping the solution in middle) at room temperature without disturbance for 10 minutes

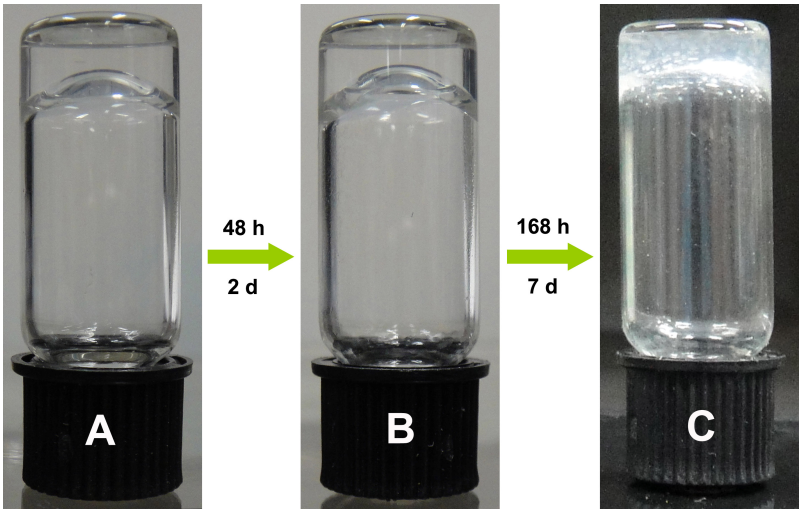



***Figure S-10***. Optical images of the gels at different time scales: A) the gel formed by treating solution of **2** (0.4 wt%) with 1 equiv. of TCEP in the PBS buffer solution after 1 hour (thixotropic), B) the gel in A) after being kept at room temperature for 48 hours (2 d, thixotropic), and C) the gel in A) after being kept at room temperature for 168 hours (7 d, not thixotropic)