Responsive peptide-based supramolecular hydrogels constructed by self-immolative chemistry

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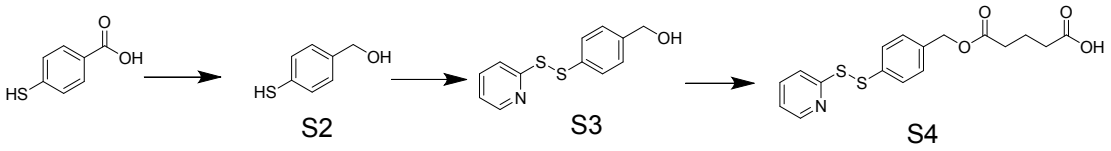
Experimental supporting information

Preparation of responsive capping groups

1、Preparation of GSH-responsive capping group compound S4:



Scheme S1. Synthetic route for the GSH-responded capping group

Compound S2: (4-Mercaptophenyl)methanol (S2) was prepared as following：A solution of 4-mercaptobenzoic acid (5 g, 32.5 mmol, 1 equiv.) in dry THF (65 mL) was added dropwise over 2 h to a suspension of $LiAlH_4$ (3.7 g, 97.4 mmol, 3 equiv.) in dry THF (65 mL) under an atmosphere of N_2 at 0 °C. After 16 h, the reaction was carefully quenched by slow addition of HCl aq. (2N) and extracted with diethyl ether (3×50 mL). The organic extractions were combined and washed with H_2O (100 mL) and brine (100 mL), dried over Na_2SO_4, and then concentrated to dryness in vacuo. The resulting residue was purification by silica gel chromatography (R_f = 0.5 ethyl acetate/petroleum ether, 1/2 *v/v*; ethyl acetate/ petroleum ether, from 1/8 to 1/3 v/v) to afford compound S2 (3.7 g, yield of 81.5%) as a white powder. δ_H (400 MHz, DMSO) 7.47 (1 H, d, *J* 8.3), 7.32 (1 H, d, *J* 8.2), 4.47 (1 H, s).

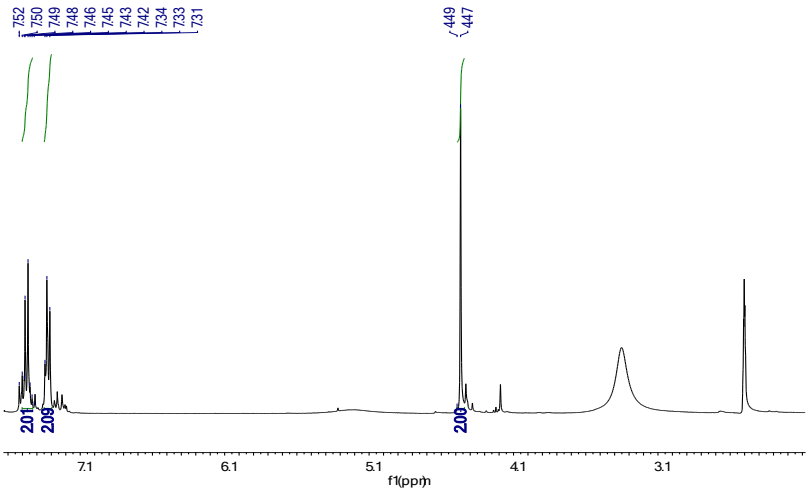


Figure S1. ^{1}H NMR spectrum of Compound S2

Compound S3 (PBMA): 4-(2-Pyridinyldithio)benzenemethanol (PBMA) was prepared as following: 4-Mercaptobenzyl alcohol (S2) (420 mg, 2 mmol) was dissolved in dry CH_2Cl_2 (2.25 mL) and mixed with a solution of 2,2′-dithiodipyridine (1320 mg, 4 mmol) in dry CH_2Cl_2 (3.25 mL). After being stirred overnight, the excess solvent was removed by evaporation, following by purification by silica gel chromatography (R_f= 0.7; ethyl acetate/ petroleum ether, from 1/10 to 1/4 *v/v*). After drying under reduced pressure, 4-(2-pyridyldithio) benzyl alcohol was obtained as a pale yellow oil (348.6 mg, yield of 70%). δ_H (400 MHz, DMSO-d_6) 8.48 (1 H, ddd, *J* 4.7, 1.7, 0.8), 7.88 – 7.77 (1 H, m), 7.75 – 7.65 (1 H, m), 7.58 – 7.47 (2 H, m), 7.33 (2 H, d, *J* 8.3), 7.27 (1H, ddd, *J* 7.4, 4.8, 0.9), 5.24 (1 H, s), 4.47 (2 H, s).

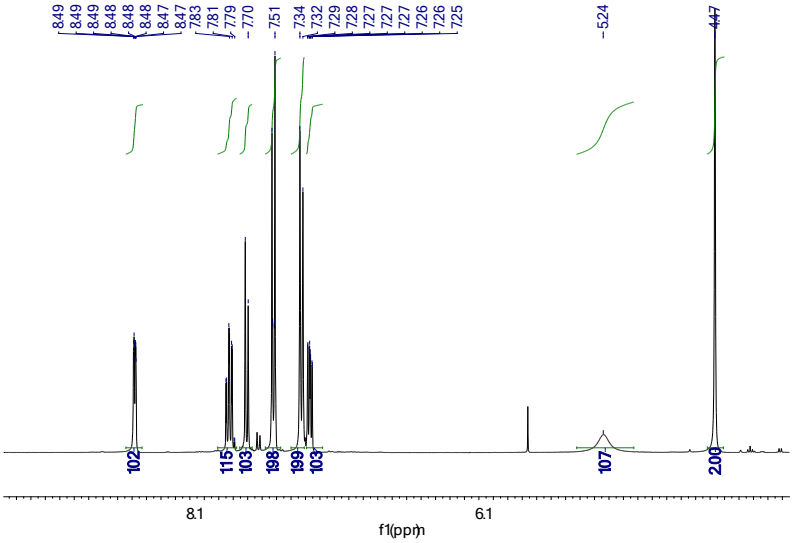
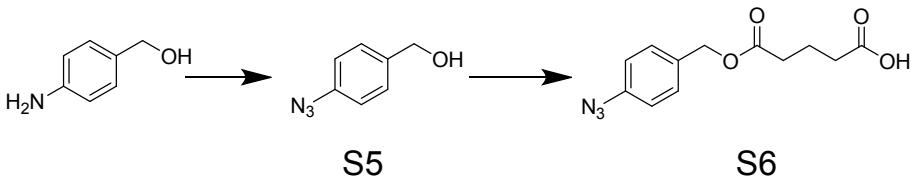


Figure S2. ^{1}H NMR spectrum of Compound S3

Compound S4 (PBMA-GA): Compound S3 (996 mg, 4 mmol) was dissolved in CH_2Cl_2 (25 mL). To this solution, triethylamine (840 µL, 6 mmol) was added within

10 min. Glutaric anhydride (500 mg, 4.4 mmol) was then added. The resulting solution was stirred at room temperature overnight. The reaction was monitored by LC-MS. The crude product was used directly for the next step.

2、Preparation of H_2S-responsive capping group compound S6:



S5 S6

Scheme S2. Synthetic route for the H_2S-responsive capping group.

Compound S5 (ABA): 4-Aminobenzylalcohol (ABA) (1.0 g, 8.12 mmol) was dissolved in hydrochloric acid (5 mL, 12 M). To this solution, sodium nitrite (840 mg, 12.18 mmol) dissolved in 20 mL of water was dropwise added within 30 min. The solution was vigorously stirred in ice-cold water. Sodium azide (2.1 g, 32.3 mmol) was batch added in. The resulting solution was stirred at room temperature overnight. The reaction was monitored by TLC. After the completion of reaction, the reaction solution was poured into saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The combined organic extractions were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel chromatography (ethyl acetate/petroleum ether = 1:3, v/v, R_f=0.65) to obtain the pure compound S5 as a yellow oil (1.45 g, 99% yield). δ_H (400 MHz, DMSO-d_6) 7.33 (1 H, d, *J* 8.4), 7.15 – 6.92 (1 H, m), 5.22 (0 H, t, *J* 5.5), 4.46 (1 H, d, *J* 5.4).

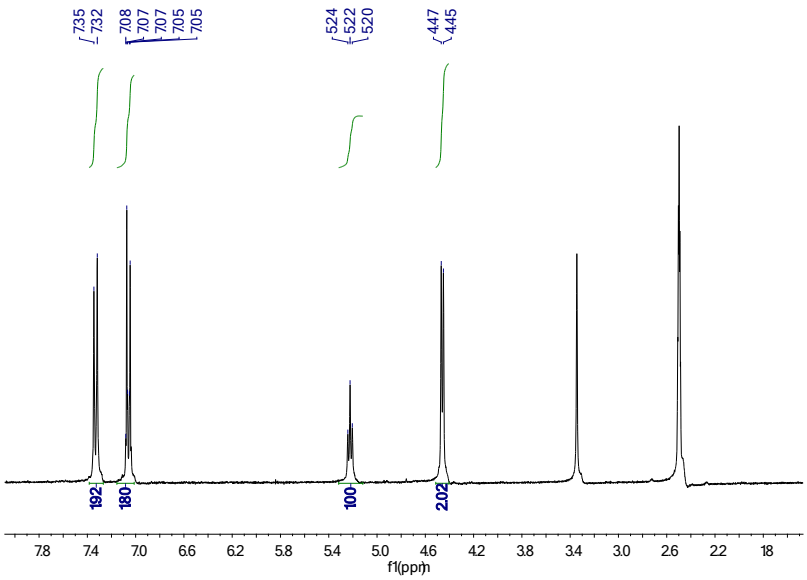


Figure S3. ^{1}H NMR spectrum of Compound S5.

Compound S6 (ABA-GA): 4-Azidebenzylalcohol (447 mg, 3mmol) was dissolved in CH_2Cl_2 (30 mL). To this solution, triethylamine (630 µL, 4.5 mmol) was added within 10min. Glutaric anhydride (376 mg, 3.3 mmol) was then added. The resulting solution was stirred at room temperature overnight. After that, the excess solvent was removed by evaporation, followed by purification by silica gel chromatography (ethyl acetate/petroleum ether/ acetic acid, 1/1/0.05 *v/v/v*, R_f= 0.4) to obtain the pure compound S6 as a yellow oil (631 mg, 80% yield). δ_H (400 MHz, DMSO-d_6) 12.10 (1 H, s), 7.39 (2 H, t, *J* 16.8), 7.25 – 6.92 (2 H, m), 5.07 (2 H, s), 2.44 – 2.30 (2 H, m), 2.25 (2 H, t, *J* 7.4), 1.74 (2 H, p, *J* 7.4).

δ C (101 MHz, CDCl3) 179.21, 172.70, 140.11, 129.92, 119.24, 119.17, 65.75, 33.12, 32.97, 32.92, 19.75, 19.49.

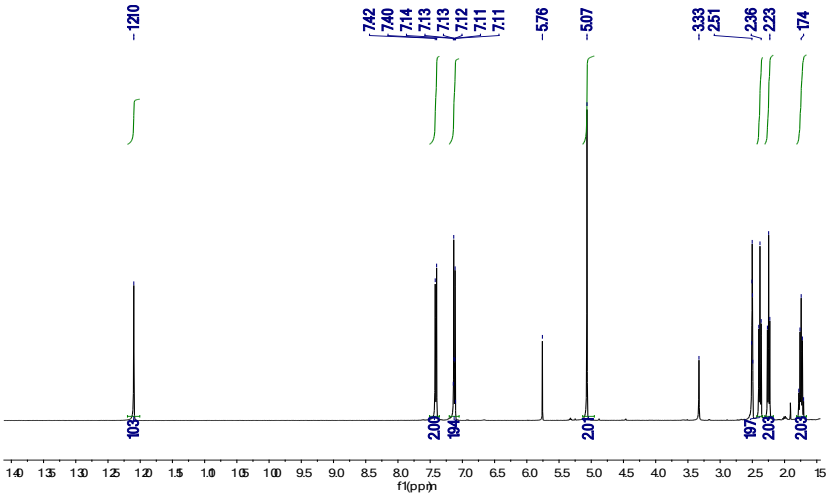


Figure S4. ^{1}H NMR spectrum of Compound S6.

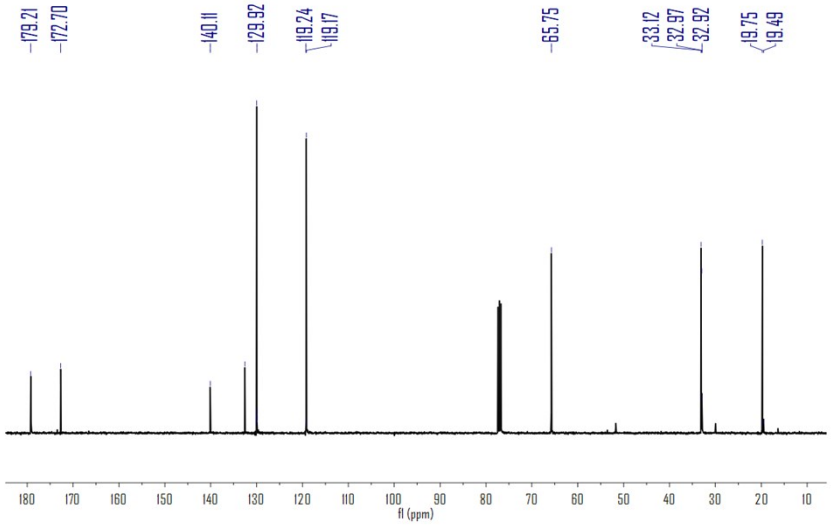


Figure S5. ^{13}C NMR spectrum of Compound S6.

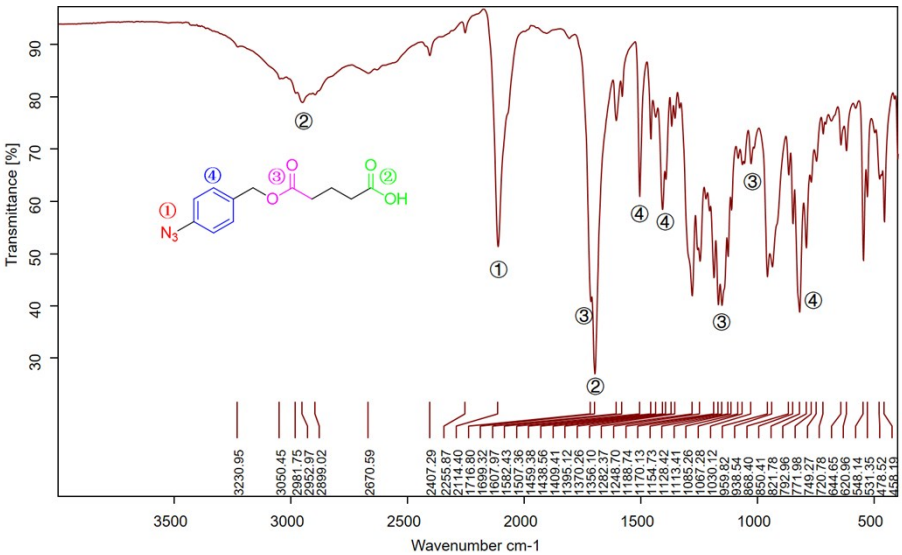
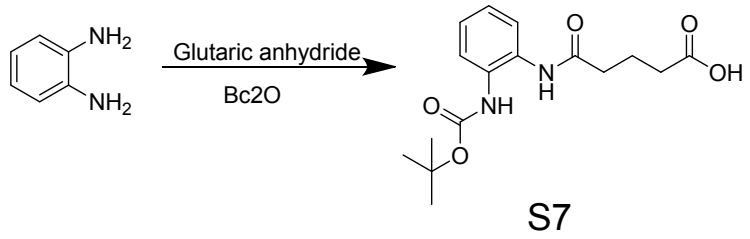


Figure S6. FT-IR spectrum of Compound S6.

3、Preparation of NO-responsive capping group compound S7:



Scheme S3. Synthetic route for the NO-responsive capping group

Compound S7 (TBAM-GA): 5-((2-((tert-butoxycarbonyl) amino) phenyl) amino)-5-oxopentanoic acid (TBAM-GA) was prepared as following: O-phenylenediamine (424 mg, 4mmol) was dissolved in CH_2Cl_2 (30 mL). To this solution, triethylamine (840 μL, 6 mmol) was added within 10min. Glutaric anhydride (500 mg, 4.4 mmol) was then added in. The resulting solution was stirred at room temperature overnight. Boc_2O (4 mmol) in CH_2Cl_2 (10 mL) was then added. The resulting solution was stirred at room temperature overnight. The reaction was monitored by LC-MS. The reaction solution was extracted with HCl (2N), and the organic phase was collected and dried over anhydrous sodium sulfate. The organic solvent was spin-dried and purified by silica gel column (DCM / MeOH / acetic acid, 30/1/0.005 *v/v/v*, R_f = 0.6) to obtain the off-white oil pure product. δ H (400 MHz, DMSO-d_6) 12.07 (1 H, s), 9.44 (1 H, s), 8.31 (1 H, s), 7.51 (1 H, d, J 7.8), 7.39 (1 H, dd, J 7.7, 1.6), 7.08 (2 H, ddd, J 12.2, 7.6, 1.7), 2.39 (2 H, t, J 7.3), 2.28 (2 H, t, J 7.4), 1.88 – 1.76 (2 H, m), 1.46 (9 H, d, J 2.4).

δ C (101 MHz, CDCl3) 177.66, 172.16, 154.54, 130.90, 129.53, 126.37, 125.39, 125.26, 124.56, 81.18, 35.58, 32.98, 28.36, 28.28, 20.64.

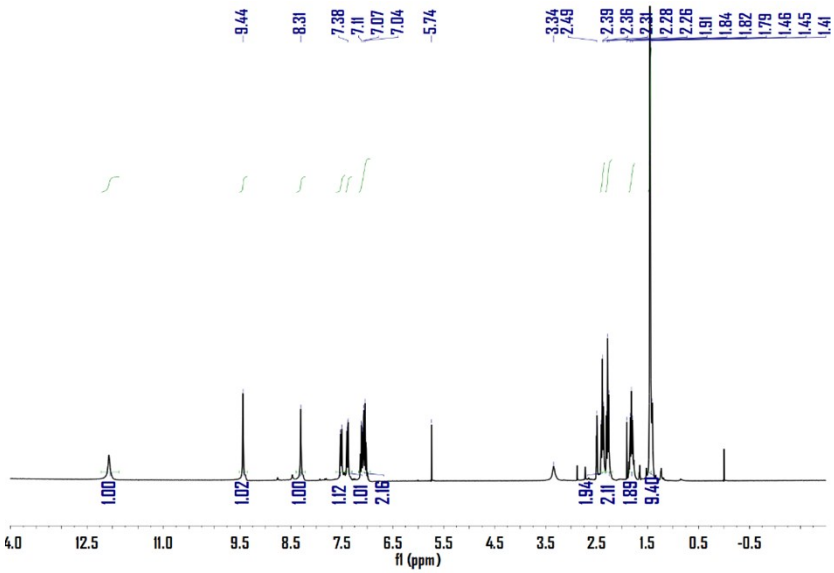


Figure S7. ^{1}H NMR spectrum of Compound S7.

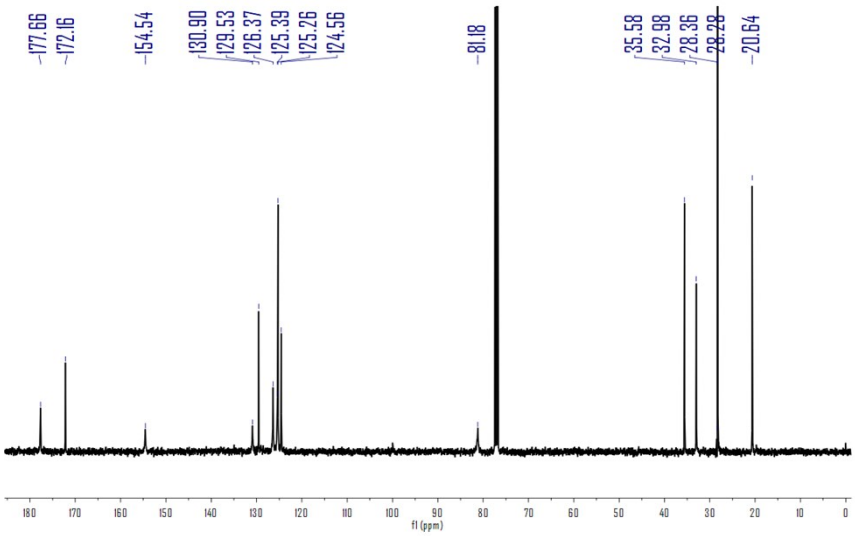


Figure S8. ^{13}C NMR spectrum of Compound S7.

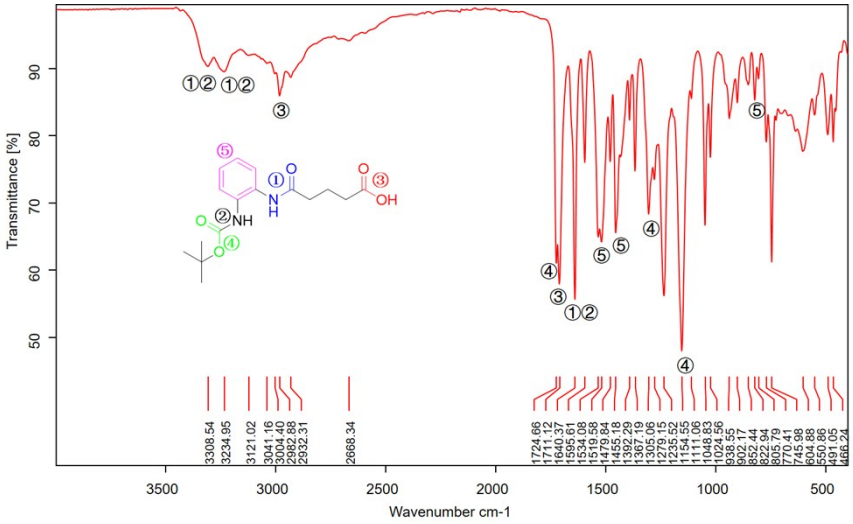


Figure S9. FT-IR spectrum of Compound S7.

Preparation of peptide derivatives: The peptide derivatives were prepared by 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 or t-butyl oxy carbonyl group. The first amino acid (Fmoc-L-Tyr(OtBu)-OH were loaded on the resin at the C-terminal with the loading efficiency about 1.0 mmol/g. 20 % piperidine in anhydrous N, N'-dimethylformamide (DMF) was used during 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'-tetramethyluraniumhexafluorophosphate (HBTU) as the coupling reagent. The growth of the peptide chain was according to the established Fmoc SPPS protocol. At the final step, the three responsive capping groups and Glutaric anhydride were used to couple

with the peptides. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 times (about 5 ml per gram of resin), followed by five steps of washing using DCM for 5 times (about 5 ml per gram of resin). The peptide derivatives were cleaved using 95% of trifluoroacetic acid (TFA) with 2.5 % of trimethyl silane (TIS) and 2.5 % of H_2O for 30 minutes. All the solutions were combined and concentrated, 20 mL ice-cold diethyl ether was then added. Afterward the supernatant was decanted and the resulting crude product was purified by high performance liquid chromatography and lyophilized. Three responsive peptide derivatives were named as PBMA-GA-Phe-Phe-Tyr (compound **1**), TBAM-GA-Phe-Phe-Tyr (compound **2**), and ABA-GA-Phe-Phe-Tyr (compound **3**) respectively. The control peptide derivative was named as GA-Phe-Phe-Tyr (compound **4**).

Characterizations of peptide derivative

Characterizations of compound 1

δ H (400 MHz, DMSO) 12.70 (1 H, s), 9.24 (1 H, s), 8.48 (1 H, d, J 4.3), 8.24 (1 H, d, J 7.6), 8.03 (2 H, dd, J 38.4, 8.5), 7.80 (1 H, dd, J 11.5, 4.0), 7.68 (1 H, d, J 8.1), 7.60 – 7.43 (2 H, m), 7.34 (2 H, dd, J 20.9, 8.2), 7.30 – 7.10 (10 H, m), 7.05 (3 H, dd, J 19.1, 7.3), 6.66 (2 H, d, J 8.3), 5.03 (2 H, s), 4.63 – 4.41 (2 H, m), 4.41 (1 H, s), 3.12 – 2.88 (3 H, m), 2.87 – 2.71 (2 H, m), 2.67 – 2.57 (1 H, m), 2.05 (4 H, dt, J 40.9, 7.3), 1.64 – 1.46 (2 H, m), 1.32 – 1.15 (1 H, m). HRMS: cacl. M^+ =820.2601, obsvd. $(M+H)^+$ =821.2692.

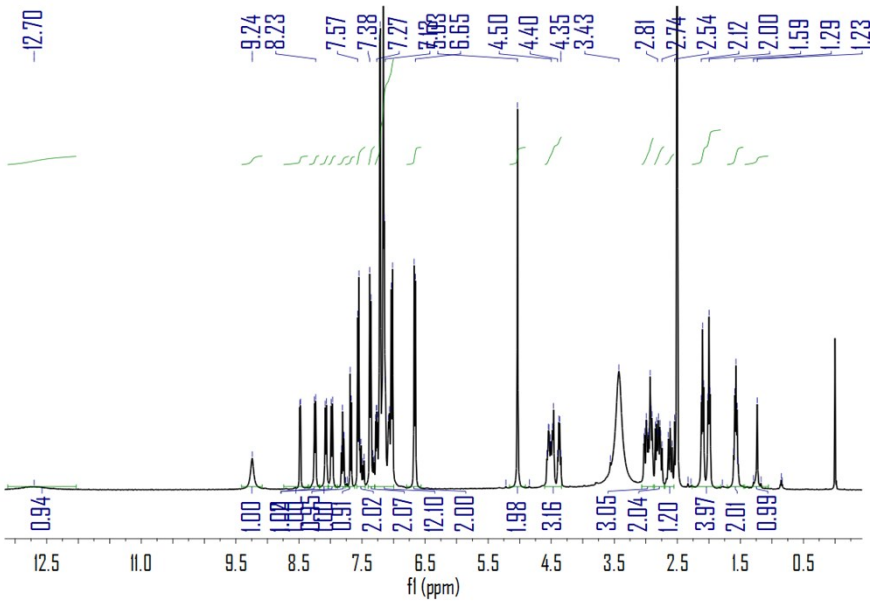


Figure S10. ^{1}H NMR spectrum of compound **1**.

The purity of compound **1** is about 96%.

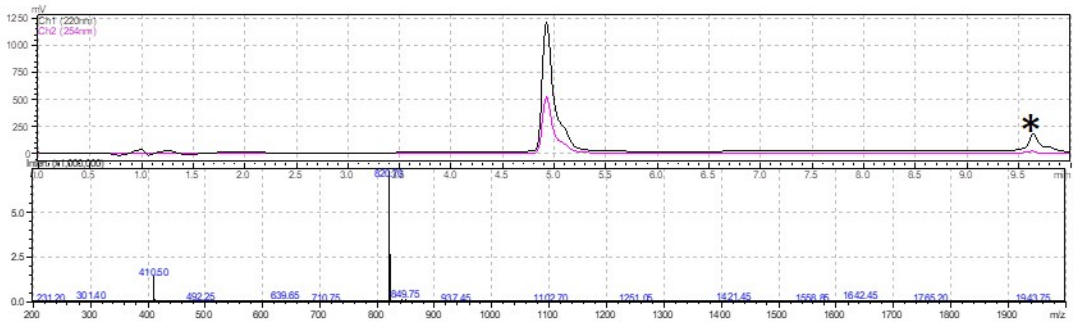


Figure S11. LC-MS spectrum of compound **1**.

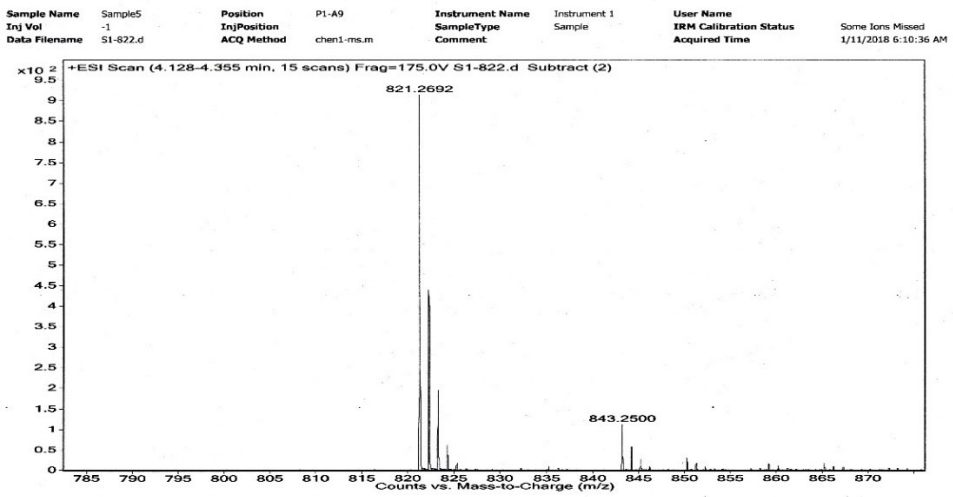


Figure S12. HR-MS spectrum of compound **1**.

Characterizations of compound 2

δ_H (400 MHz, DMSO) 12.77 (1 H, s), 9.69 (1 H, s), 9.25 (1 H, s), 8.29 (1 H, m), 8.10 (2 H, m), 7.77 (1 H, dd, *J* 6.1, 3.1), 7.53 (1 H, dd, *J* 6.1, 3.1), 7.17 (8 H, m), 7.03 (2 H, m), 6.66 (2 H, dd, *J* 8.4, 2.9), 4.52 (2 H, ddd, *J* 22.7, 12.8, 6.3), 4.38 (1 H, dd, *J* 10.5, 5.4), 2.95 (3 H, m), 2.80 (2 H, m), 2.65 (1 H, ddd, *J* 13.9, 10.3, 6.9), 2.26 (1 H, m), 2.10 (1 H, m), 1.95 (1 H, m), 1.69 (1 H, dd, *J* 13.7, 8.5), 1.43 (1 H, d, *J* 30.1). HRMS: cacl. M^+ =679.3006, obsvd. $(M+H)^+$ =680.3098.

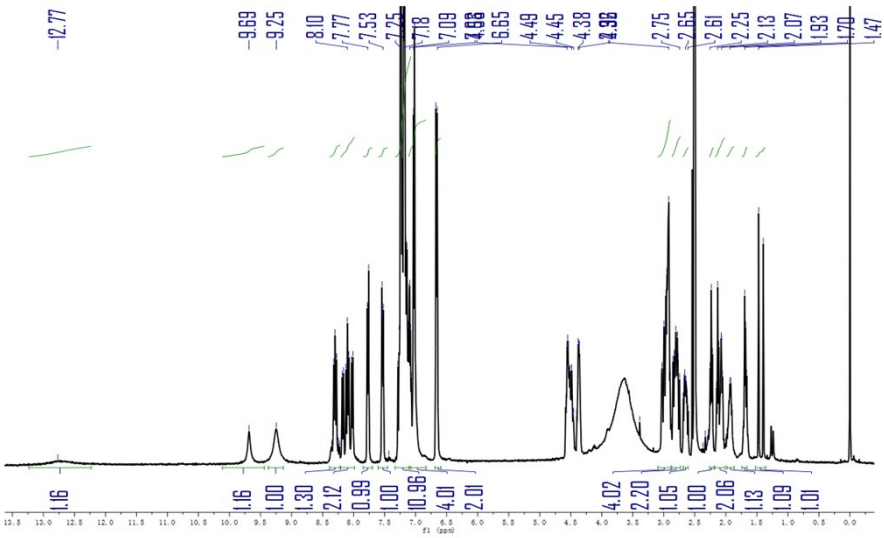


Figure S13. ^{1}H NMR spectrum of compound **2**.

The purity of compound **2** is about 95%.

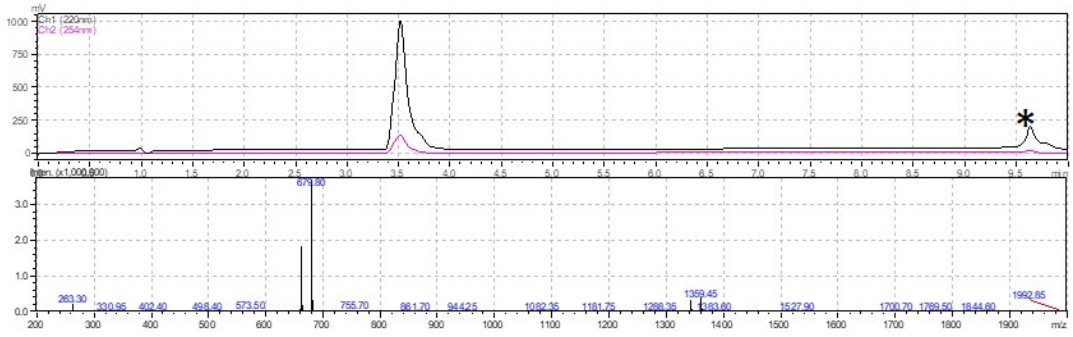


Figure S14. LC-MS spectrum of compound **2**.

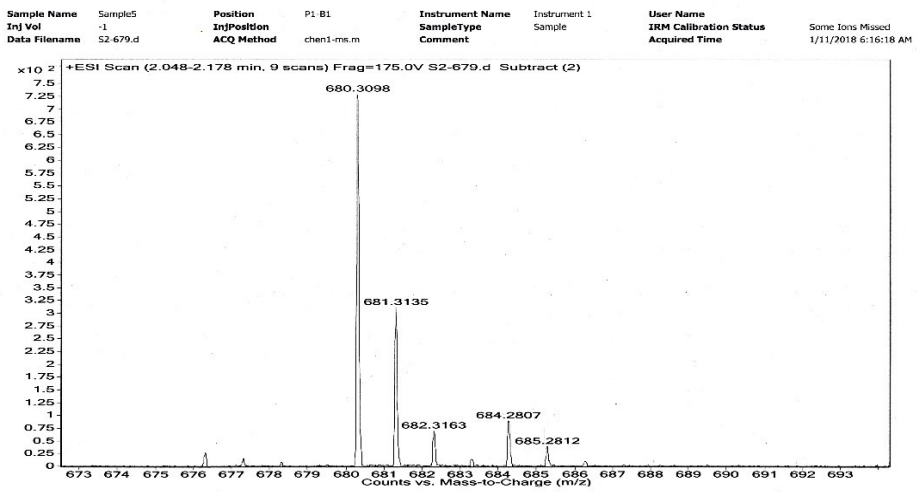


Figure S15. HR-MS spectrum of compound **2**.

Characterizations of compound 3

δ_H (400 MHz, DMSO) 12.73 (1 H, s), 9.23 (1 H, s), 8.23 (1 H, d, *J* 7.7), 8.01 (2 H, dd, *J* 33.0, 8.5), 7.40 (2 H, d, *J* 8.4), 7.14 (10 H, m), 7.01 (2 H, t, *J* 9.5), 6.63 (2 H, t, *J* 16.6), 5.04 (2 H, s), 4.51 (2 H, m), 4.37 (1 H, dd, *J* 13.4, 7.8), 2.95 (3 H, m), 2.78 (2 H, m), 2.62 (1 H, dd, *J* 13.7, 10.7), 2.12 (2 H, m), 2.00 (2 H, t, *J* 7.2). HRMS: cacl. M^+ =720.2908, obsvd. $(M+H)^+$ =721.2988.

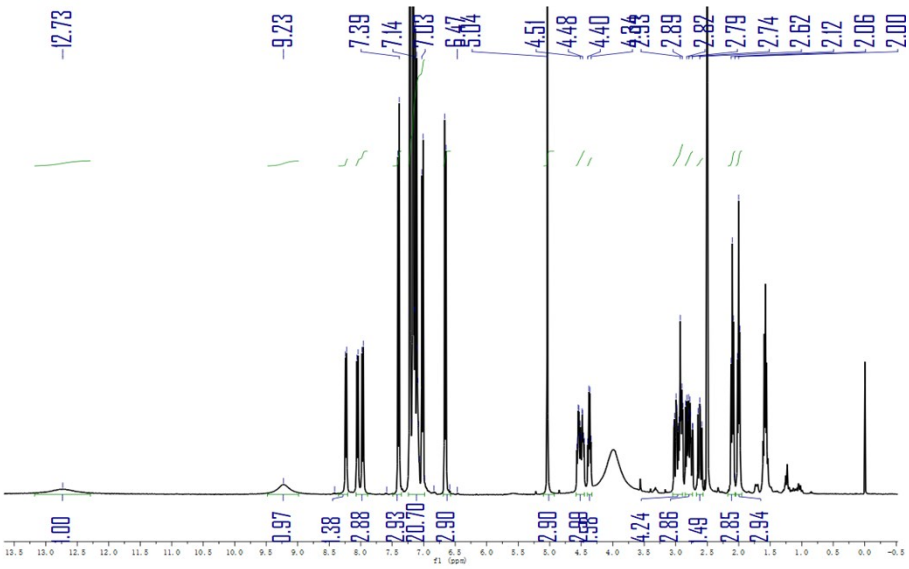


Figure S16. [1]H NMR spectrum of compound **3**.

The purity of compound **3** is about 99%.

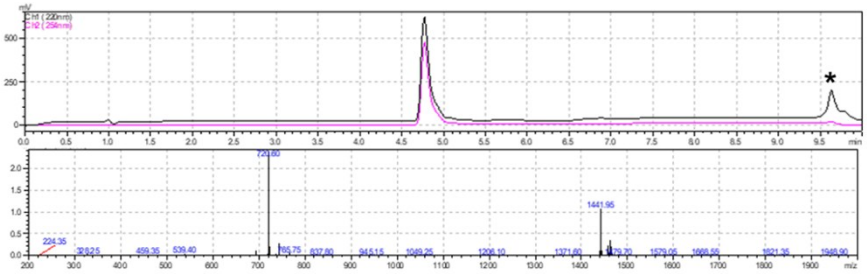


Figure S17. LC-MS spectrum of compound **3**.

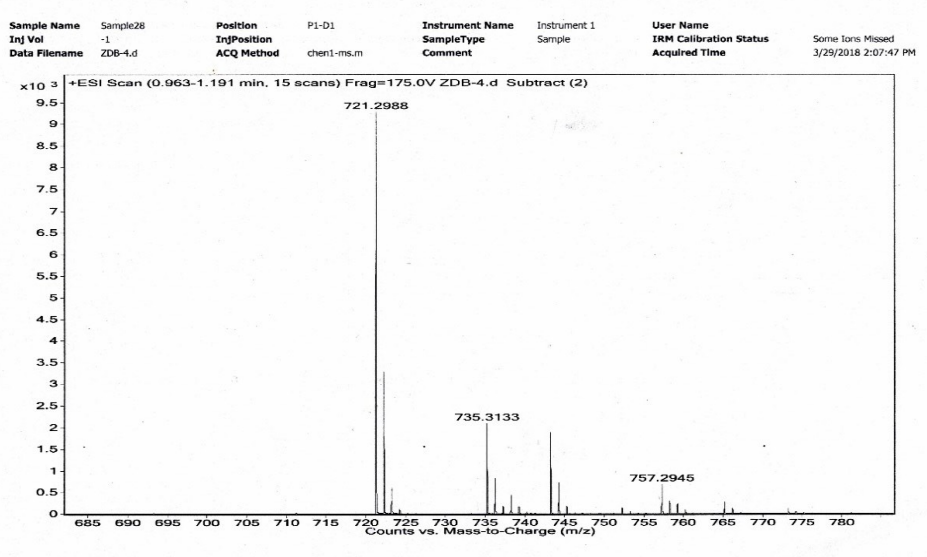


Figure S18. HR-MS spectrum of compound **3**.

Characterizations of compound 4

δ_H (400 MHz, DMSO) 12.19 (2 H, s), 9.23 (1 H, s), 8.23 (1 H, d, *J* 7.7), 8.05 (2 H, d, *J* 8.3), 7.96 (2 H, d, *J* 8.6), 7.18 (10 H, m), 7.02 (2 H, d, *J* 8.4), 6.66 (2 H, d, *J* 8.4), 4.51 (2 H, m), 4.37 (1 H, dd, *J* 13.4, 7.8), 2.96 (3 H, m), 2.79 (2 H, m), 2.64 (1 H, m), 2.01 (4 H, m), 1.54 (2 H, p, *J* 7.3). HRMS: cacl. M^+ =589.2424, obsvd. $(M+H)^+$ =590.2495.

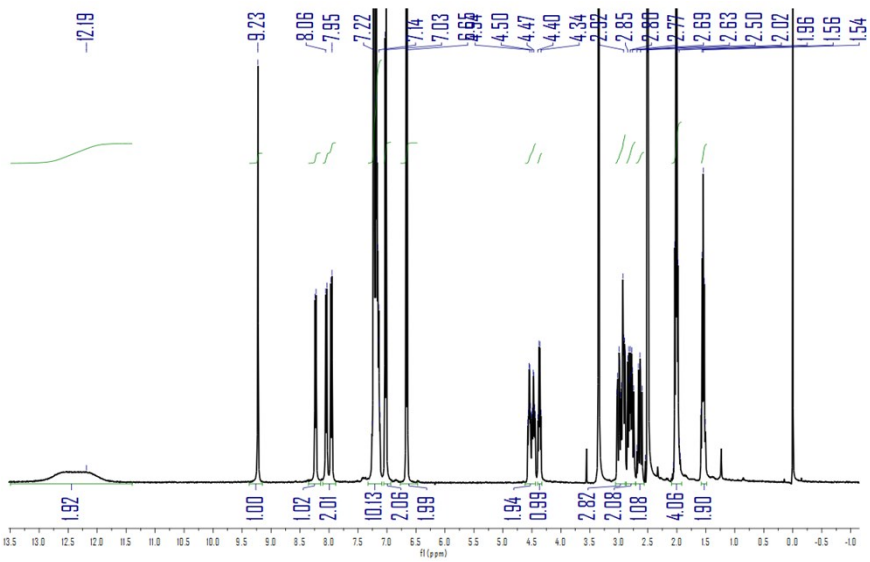


Figure S19. ^{1}H NMR spectrum of compound **4**.

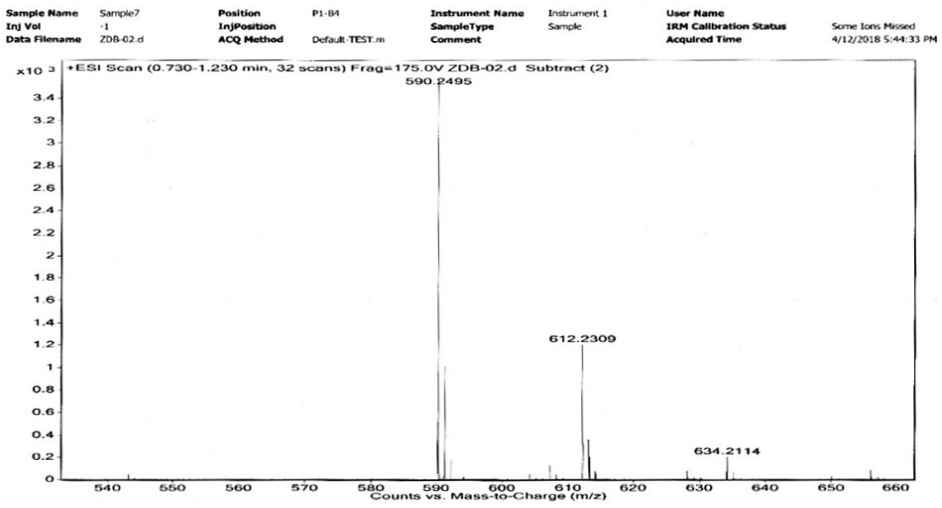


Figure S20. HR-MS spectrum of compound **4**.

Hydrogel formation and Rheology

Two mg of compound **1** was suspended of in 400 μL of PBS and 5 μL Na_2CO_3 aqueous solution (1M) was added to adjust the pH to 7.4. The mixture was heated to around 90 ℃ to make a clear solution. Hydrogel would form when the solution was cooled back to room temperature. Using the same procedure, we prepared H_2S-responsive hydrogel from compound **3** (the only difference was that pH needed to be adjusted to 7.4), otherwise there would be a precipitation at the concentration of 0.5 wt%. Compound **2** (2 mg) and 4 μL. of Na_2CO_3 aq (1M) (to adjust the pH to 7.0) were dissolved in 400 μL of PBS *via* a heating–cooling process to make a clear solution. The gel would form after

standing for 24 hours. We tested the critical gelation concentration (CGC) of various peptides derivatives. We used a rheometer to characterize the resulting hydrogels containing 0.5 wt% of the peptides using 25 mm parallel plates and at the gap of 450 μm.

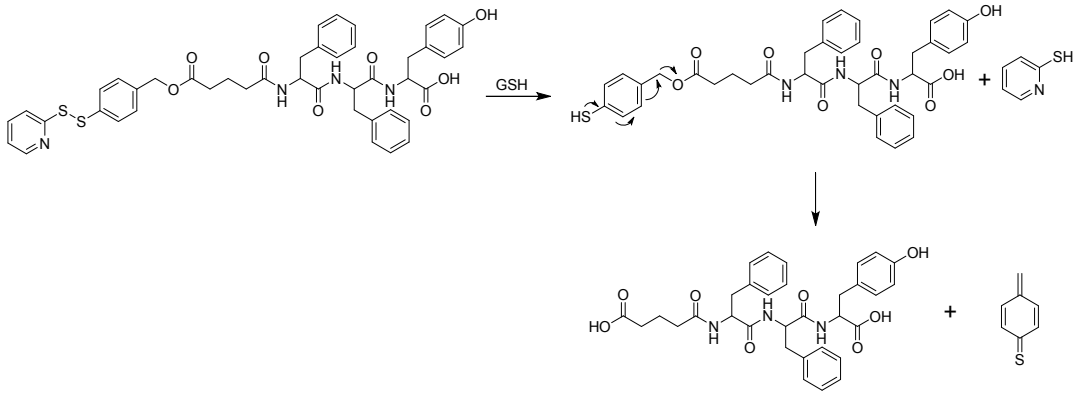
Cell Culture

LO2 cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). LO2 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% v/v fetal bovine serum (FBS), 100 U/mL penicillin and 100 g/mL streptomycin. Cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2.

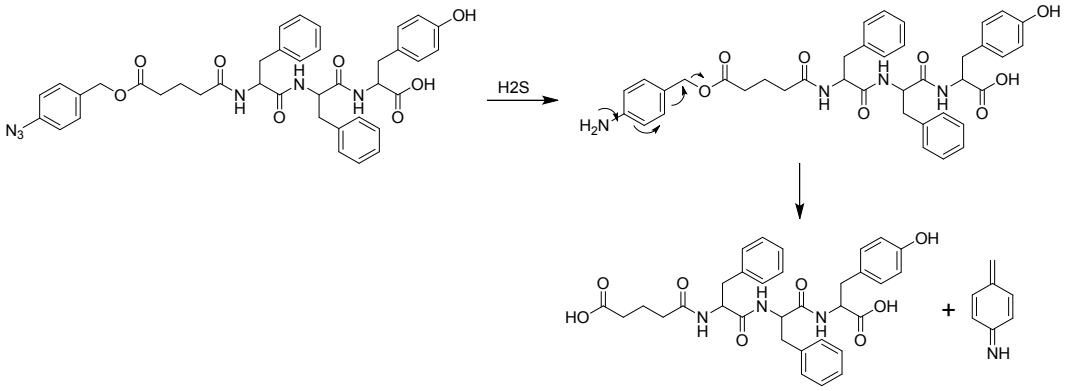
Responsive mechanism of the hydrogels

1、 GSH-responsive hydrogel and H_2S-responsive hydrogel

The glutathione can reduce disulfide bond to thiol groups, and hydrogen sulfide can reduce azide groups to amino groups. An electron donating group will cause the loss of the "self-immolative" group. At a macroscopic level, the hydrogel becomes a solution. The reaction mechanism is shown as following:



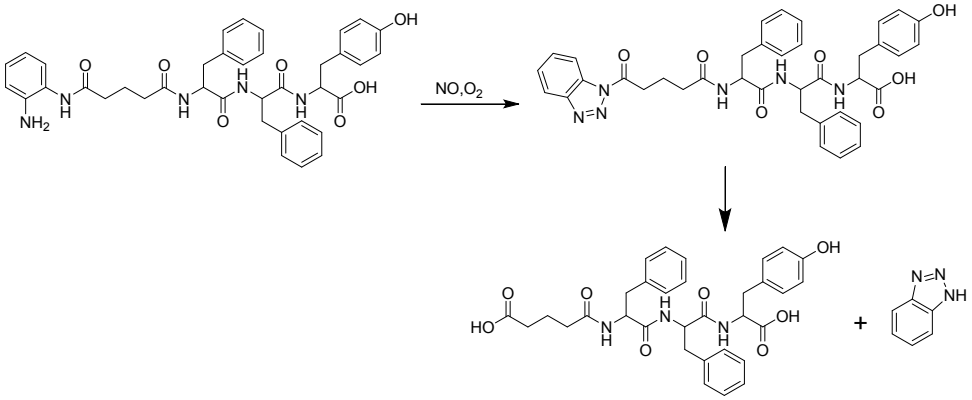
Scheme S4. The response mechanism for GSH-triggered gel-sol phase transition.



Scheme S5. The response mechanism for H_2S-triggered gel-sol phase transition.

2、 NO-triggered gel-sol phase transition

The O-phenylenediamine amide compounds can react with NO to yield an amide-substituted benzotriazole intermediate that is sensitive to hydrolysis in an aqueous solution to form benzotriazole and residual carboxyl groups. We use LC-MS to monitor the reaction. As shown in Figure S6, compound **1** can react with NO rapidly to form intermediates, and the intermediates undergo relatively slow spontaneous hydrolysis, so we can detect the formation of intermediates using LC-MS.



Scheme S6. The response mechanism for NO-triggered gel-sol phase transition.

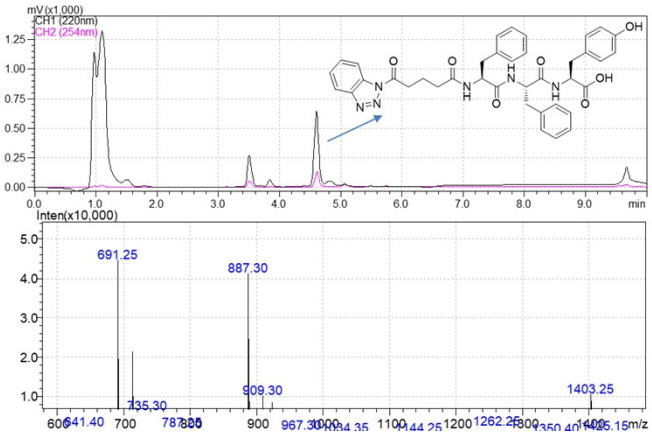


Figure S21. LC-MS traces of that solution of peptide reacted with NO.

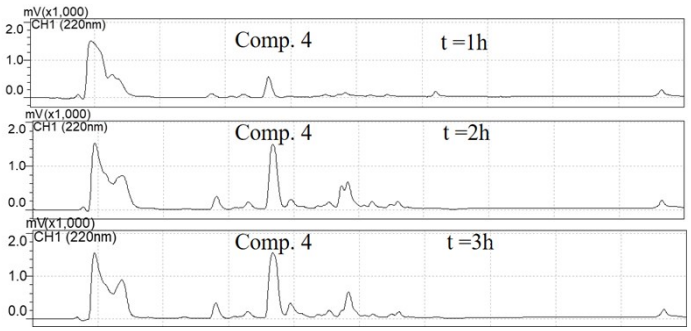


Figure S22. LC traces of hydrogels at different time points after addition of GSH (8 eq).

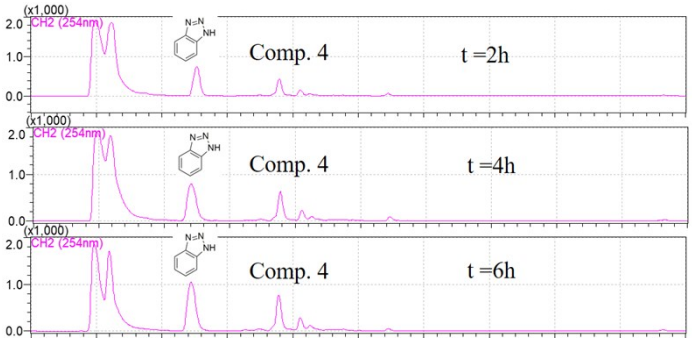


Figure S23. LC traces of hydrogels at different time points after addition of SNP (0.1M).

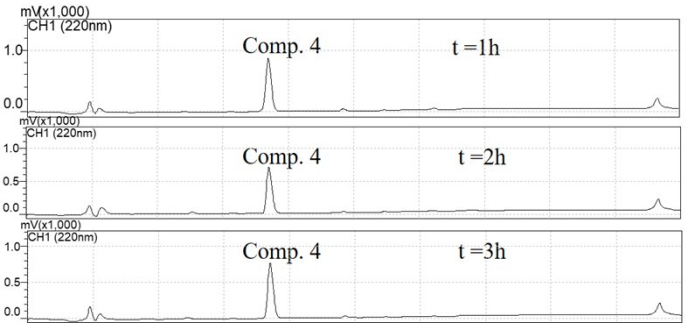


Figure S24. LC traces of hydrogels at different time points after addition of H_2S (10 eq).

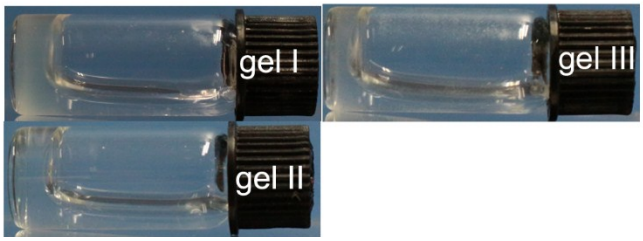


Figure S25. Optical images of hydrogels contacting with equal volume of PBS for 48h.

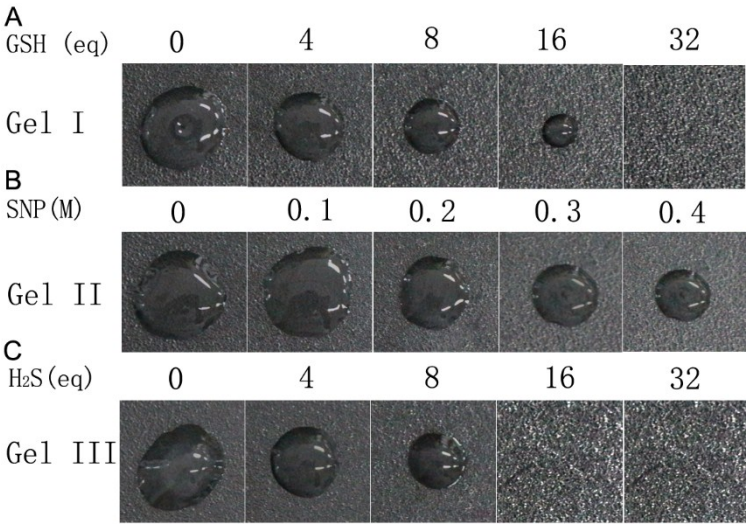


Figure S26. Optical images of the spot assay to show responsiveness of different gels (150μL) to different concentration of small molecules in a 50μL solution after two hours: (A) gel I to GSH in PBS (pH = 7.4), (B) gel II to SNP in PBS (pH = 7.0), and (C) gel III to H_2S in PBS (pH = 7.4). The control was the gel without adding the solution of corresponding trigger molecule.

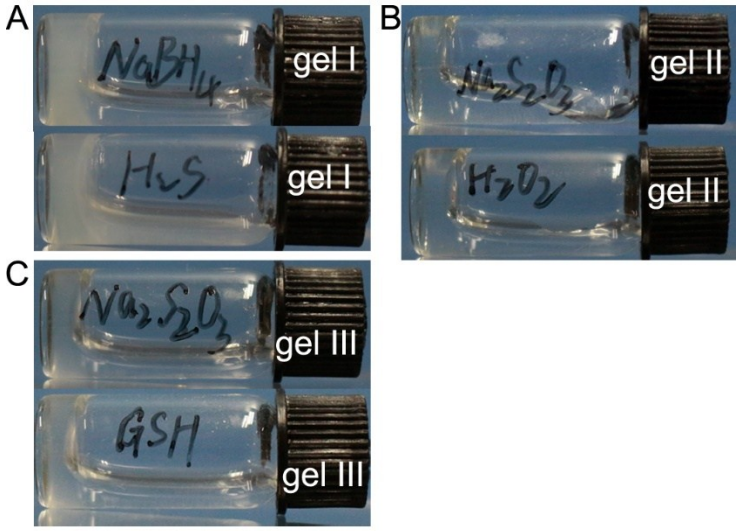


Figure S27. Optical images of (A) gel I contacting with $NaBH_4$ solution (pH= 7.4, 10 mM) or H_2S solution (pH= 7.4, 10 mM) for 12h. (B) gel II contacting with $Na_2S_2O_3$ solution (pH= 7.0, 10mM) or H_2O_2 solution (pH= 7.0) for 12h. (C) gel III contacting with $Na_2S_2O_3$ solution (pH= 7.4, 10mM) or GSH solution (pH= 7.4, 10 mM) for 12h.

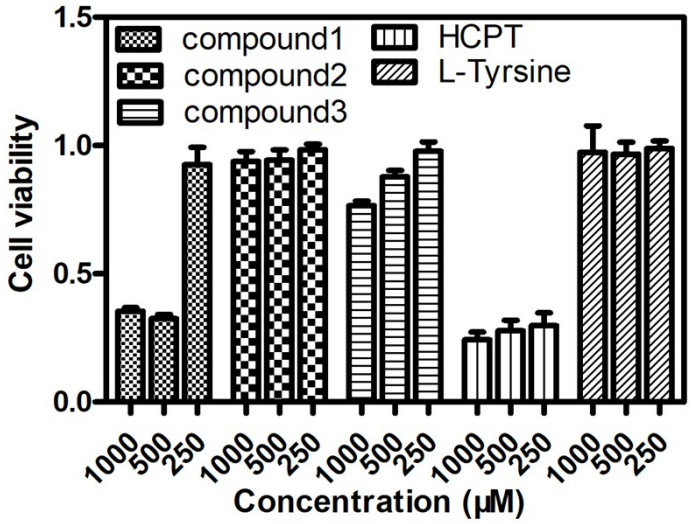


Figure S28. Cell viability of LO2 cells in the presence of compound **1**, **2**, **3**, HCPT and L-Tyrosine in which HCPT and L-Tyrosine were as positive and negative controls, respectively.