Supporting Information

Preparation and Characterization of Self-healing Smart Hydrogel Based on Polypeptides with Dual Response to Light and Hydrogen Peroxide

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Synthesis of the N-(p-nitrophenoxycarbonyl)-γ-o-nitrobenzyl-L-glutamate (NPNBLG)

Briefly, o-nitrobenzyl alcohol (1.53 g, 10 mmol, 1.0 equiv.) was dissolved in dichloromethane (30 mL),thenphosphorus tribromide (1.05 mL, 1.1 equiv) was added at ice bath. The mixture was then stirred at room temperature and monitored by TLC for 6 h. After that, the reaction mixture was washed with saturated brine, dried over MgSO4, and the solvent was removed under reduced pressure. Then the product was dried overnight in a vacuum oven at room temperature, affording o-nitrobenzyl bromide as a yellowish solid for using without further purification.

In a 250-mL round bottom flask, N,N,N',N'-tetramethylguanidine (11 mL, 87 mmol) was added slowly to a stirredL-glutamic acid andL-glutamic acid copper (II) complex (30.8 g, 86 mmol) in DMF (40 mL) and distilled water (6 mL). The mixture turned to dark blue. After all solids were dissolved (~2h), additional DMF (31 mL) was added. Then, o-nitrobenzyl bromide (25 g, 90 mmol) was added to the above solution in one portion. The reaction solution became darker and was kept at 40 °C for 38 h. After that, acetone (500 mL) was added to the mixture and stirred until a fine precipitate was obtained (~1 h). The violet solid was collected by filtration, followed by addition of freshly prepared EDTA (20 g)/sodium bicarbonate (10 g) aqueous solution (150 mL) and further stirring for 24 h. The product was collected by filtration and washed with DI water. Further purification was performed by recrystallizing from H2O/isopropanol.

Obtained 15.1 g (yield: 49%) ^{1}H NMR [D2O/DCl (1wt%), δ, ppm]: 8.03 (d, 1H), 7.65 (t, 1H), 7.48~7.55 (m, 2H), 5.39 (s, 2H), 4.05 (t, 1H), 2.65 (t, 2H), 2.18 (m, 2H). ^{13}C NMR [D2O/DCl (1wt%), δ, ppm]: 24.66, 29.30, 51.80, 60.77, 63.84, 124.70, 129.20, 130.64, 134.36, 170.95, 173.95.

To a solution of γ-o-nitrobenzyl-L-glutamate (2.82 g, 10 mmol) in THF (50 mL), p-nitrophenylchloroformate (2.03 g, 10 mmol) was added at room temperature and the mixture was stirred at 40 °C for 24 h. The solvent was removed under reduced pressure, 50 mL DCM was added. Then the solution was washed with distilled water and a saturated NaCl aqueous solution, dried over anhydrous MgSO4, filtered, and concentrated by a rotary evaporator. The resulting solid residue was fractionated by column chromatography using a mixed solvent of ethyl acetate/n-hexane (1/1 v/v) to obtain NPNBLG as a white solid (1.6 g, Yield: 35%) ^{1}H NMR [CD3OD, δ, ppm]: 8.26 (d, 2H), 8.11 (d, 1H), 7.70 (m, 2H), 7.70 (m, 1H), 5.52 (s, 2H), 4.32 (m, 1H), 2.65 (t, 2H), 2.35 (m, 1H) 2.10 (m, 1H). ^{13}C NMR [DMSO, δ, ppm]: 62.55, 115.77, 124.75, 125.90, 128.95, 131.60, 134.05, 139.45, 163.75, 171.95.

Synthesis of the ((p-nitrophenoxy)carbonyl)-l-methionine

To a solution of L-methionine in THF, p-nitrophenyl chloroformate was added at room temperature and the mixture was stirred at 40 °C for 24 h. The solvent was removed under reduced pressure and dichloromethane was added. Then, the solution was washed with distilled water and a saturated NaCl aqueous solution was dried over anhydrous $MgSO_4$, filtered, and concentrated with a rotary evaporator. The resulting solid residue was fractionated by column chromatography using a mixed solvent of ethyl acetate/n-hexane (1/1 v/v) to obtain ((p-nitrophenoxy) carbonyl)-l-methionine as a white solid (yield: 45%). ^{1}H NMR [CD_3OD, d, ppm]: 8.26 (d, 2H), 7.36–7.60(d, 2H), 5.90 (d, 1H), 4.64 (m, 1H), 2.66 (m, 2H), 2.32 (m, 1H) 2.15(m, 4H).

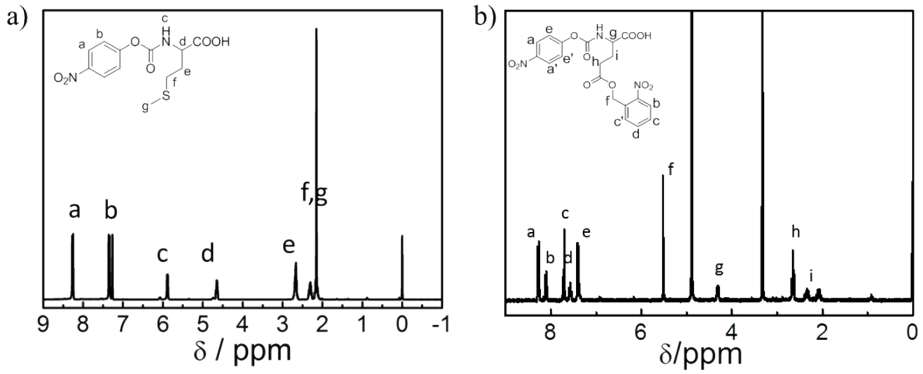

Figure S1: ^{1}H NMR spectra of monomer N-(p-nitrophenoxycarbonyl)-L-methionine and monomer N-(p-nitrophenoxycarbonyl)-γ-o-nitrobenzyl-L-glutamate.

Characterization of the polymer

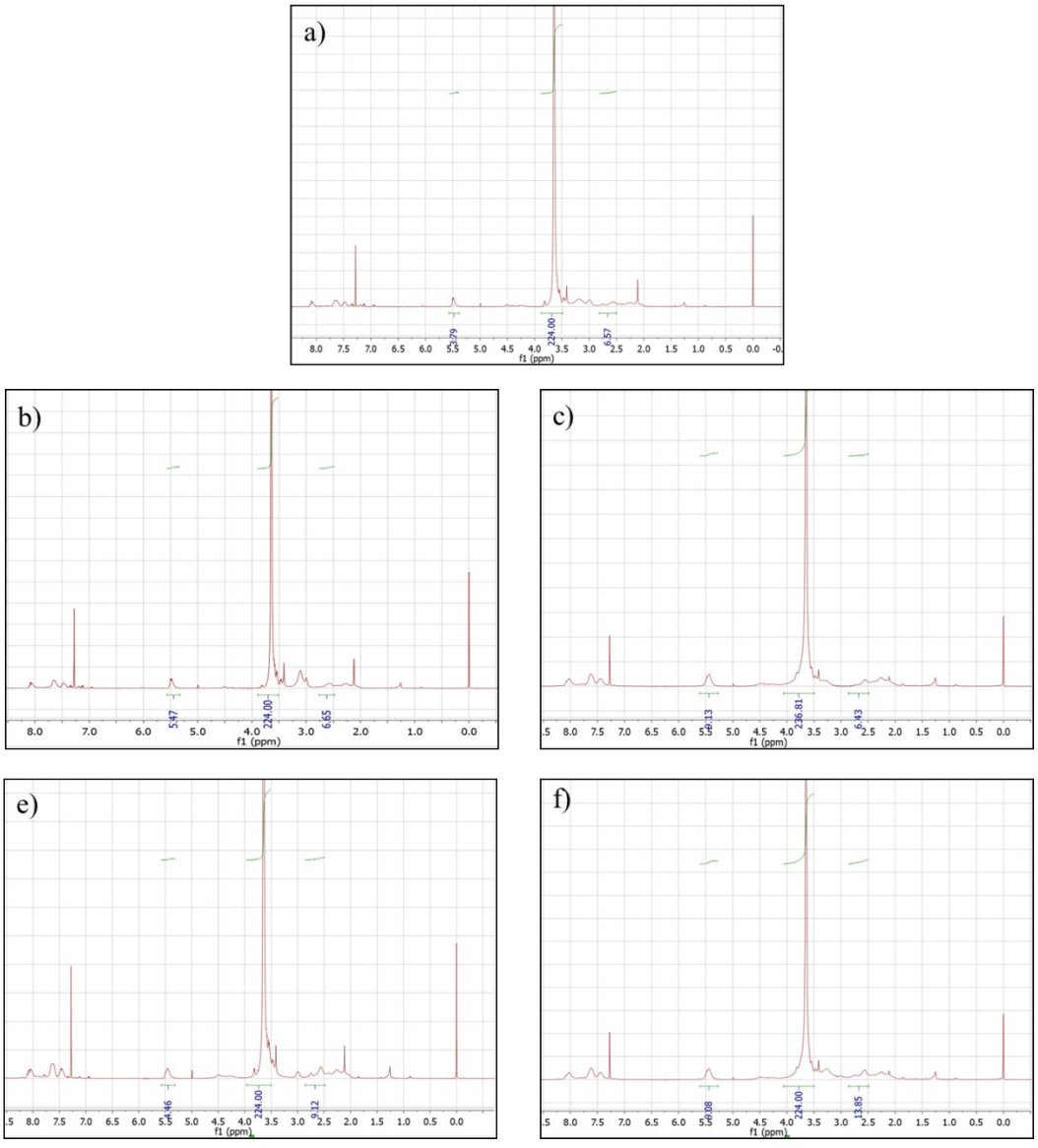

Figure S2: The ^{1}H NMR of five polypeptide-PEG conjugates in CDCl3. a) t-PEG$_{56}$-

b-P ($NBLG_2$-co-$Meth_3$), b) t-PEG_{56}-b-P ($NBLG_2$-co-$Meth_5$), c) t-PEG_{56}-b-P ($NBLG_3$-co-$Meth_3$), d) t-PEG_{56}-b-P ($NBLG_5$-co-$Meth_3$), e) t-PEG_{56}-b-P ($NBLG_5$-co-$Meth_7$).

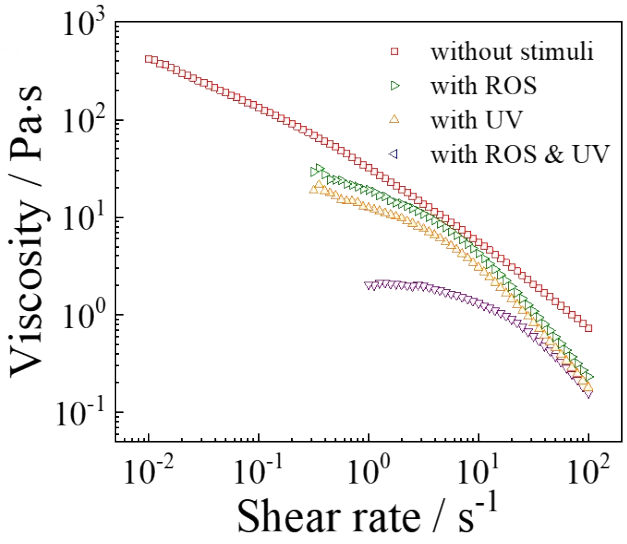

Figure S3: Shear thinning properties of hydrogel (t-PEG56-b-P (NBLG5-co-Meth7) was performed with different stimuli at 37°C: without stimuli, with ROS (hydrogen peroxide), with UV irradiation, and with UV irradiation and ROS.

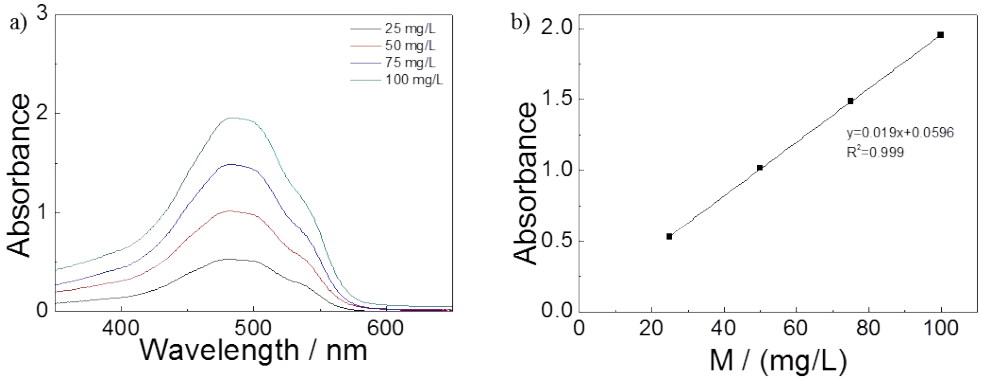

Figure S4: a) UV-vis absorbance spectra of different concentrations Dox(25mg/L,50 mg/L, 75mg/L, 100mg/L) in PBS buffer (pH=7.4, 100 mM). b) The absorbance intensity at 482nm recorded for different Dox concentrations.