Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting information

Palladium-Bridged Polymers as CO-Biosignal-Responsive, Self-Healing Hydrogels

Miaomiao Xu, Xiang Hao, Zhiwei Hu, and Qiang Yan*

State Key Laboratory of Molecular Engineering of Polymers, Fudan University, Shanghai 200433, China.

Content

1. Materials and Methods

Materials

Methyl 4-(bromomethyl) benzoate (Sigma-Aldrich, 98%), dimethylamine (J&K, 2M in THF), lithium aluminum hydride ($LiAlH_4$, J&K, 95%), methacryloyl chloride (Admas, 90%), ethyl diisopropylamine (DIPEA, Sigma-Aldrich, 99%), 2,2'-azoisobutyronitrile (AIBN, J&K, 99%), palladium acetate (Sigma, 98%), glycine (Sigma-Aldrich, 98%), sodium ethoxide (Sigma-Aldrich, 95%), tricarbonyldichlororuthenium(II) dimer (Sigma, 98%), glutathione (Sigma-Aldrich, 98%), hydrogen peroxide (Sigma-Aldrich, 30 wt% in H_2O), sodium hypochlorite (Sigma-Aldrich, 14.5% available chlorine), sodium nitrite (Sigma-Aldrich, 97%) were used as received. All solvents were used as received.

Methods

Nuclear magnetic resonance (NMR). Nuclear magnetic resonance (NMR) was taken by AVANCE III HD 400 MHz of Bruker BioSpin International with $CDCl_3$ as the solvent for characterization of the small molecule structures and CD_3CN as the solvent for characterization of the polymer structures. Solid-state ^{1}H, ^{13}C and ^{15}N NMR spectroscopy were used by FAST MAS solid-state NMR HD 800 MHz of JEOL-800 for the characterization of Pd-bridged hydrogel cross-linking structure.

Mass Spectroscopy (MALDI-TOF). The exact molecular weight of the functional monomer was taken on an AB SCIEX 5800 instrument for the analysis of functional monomers and 2-[(2E)-3-(4-tert-Butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB) was used as the matrix during the analysis of the mass.

UV-Vis Spectroscopy (UV-Vis). The UV-Vis absorption spectra of the hydrogel samples at different stimulus conditions for drug releasing were recorded by using an Agilent Cary-60 UV-Vis spectroscopy with a 1 cm cuvette.

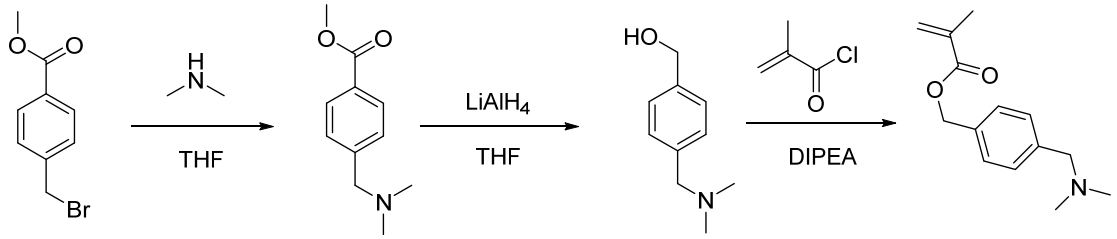
Rheological measurements. Rheological measurements were conducted on a HAAKE Par Rotary Rheometer (HAAKE MARS III). The storage modulus (G') and loss modulus (G") were measured at an angular frequency of 1 rad/s within a linear range of viscoelasticity. To investigate the recovery properties of the samples in response to

applied shear forces, the samples were placed between the para-plate and the platform with special care to avoid evaporation of water. We used the following programmed procedure (applied shear force, expressed in terms of strain; duration in parentheses): 1000% (120 s)→10%(90 s) →1000% (120 s)→10%(90 s)→1000% (120 s)→10%(90 s) →1000% (120 s)→10%(90 s)) →1000% (120 s)→10%(90 s).

Gel Permeation Chromatography (GPC). The molecular weight and the molecular weight distribution of all polymer samples were measured on a system of multiangle laser light scattering. The system is equipped with a Waters degasser, a Waters 515 HPLC pump, a Wyatt Optilab DSP differential refractometer and a Wyatt miniDAWN detector. HPLC-grade dimethylformamide (DMF) with 0.1 M LiBr was used as eluent at a flow rate of 1.0 mL/min at 30 ºC and poly (ethylene oxide) as a standard reference. The polymer solution was filtered before the GPC measurements was conducted.

2. Synthesis and Preparation

2.1 Synthesis of Carbon Monoxide (CO)-Responsive Functional Monomer



Scheme S1. Synthetic Route of Carbon Monoxide (CO)-Sensitive Functional Monomer, *p*-Dimethylamine methyl benzyl methacrylate **(BMA)**.

Synthesis of methyl 4-((dimethylamino)methyl) benzoate.

To a solution of methyl 4-(bromomethyl) benzoate (2.30 g, 10.0 mmol) in THF (10 mL) was added dimethylamine (2 M THF solution, 10 mL, 20 mmol) at 0 ^{o}C. The mixture was stirred at room temperature for 16 h. The reaction mixture was separated with saturated $NaHCO_3$ and ethyl ester. The aqueous phase was extracted in ethyl ester and the combined organic layer was washed with brine, and dried over Na_2SO_4, and then the organic phase was concentrated under reduced pressure. The residue was purified by silica gel column chromatography with CH_2Cl_2/CH_3OH at 20:1 (v/v) to give the product (yield: 88%, **Fig. S1-S2**).

^{1}H NMR (δ, ppm, $CDCl_3$): 8.00 (m, 2H, benzene); 7.40 (d, 2H, benzene); 3.91 (s, 3H, -OC**H**$_3$); 3.49 (s, 2H, -NC**H**$_2$); 2.26 (s, 6H, -N(C**H**$_3$)$_2$).

MALDI-TOF Mass Spectrum (m/z): calcd. $C_{11}H_{15}NO_2$ (M-H$^+$), 193.11; found, 192.15.

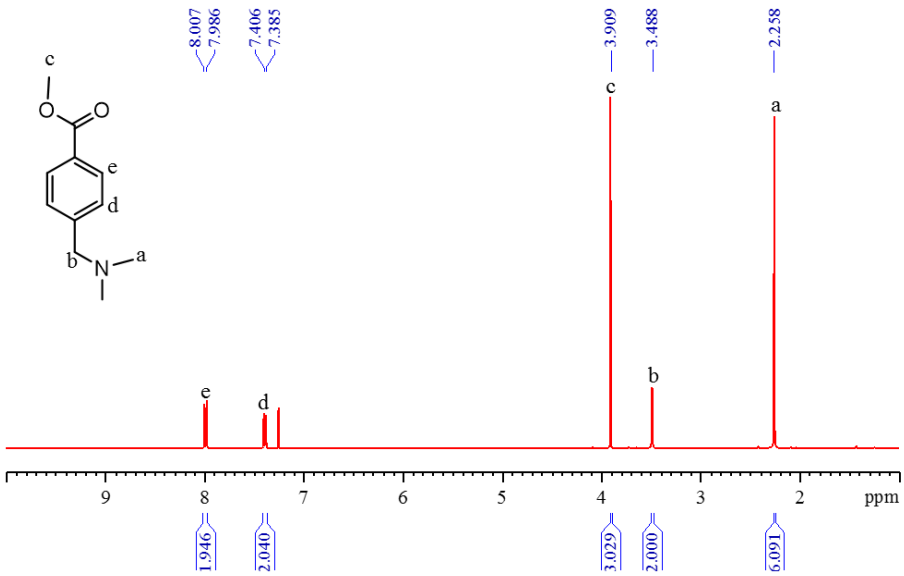


Fig. S1. ^{1}H NMR spectrum for methyl 4-((dimethylamino)methyl) benzoate in $CDCl_3$.

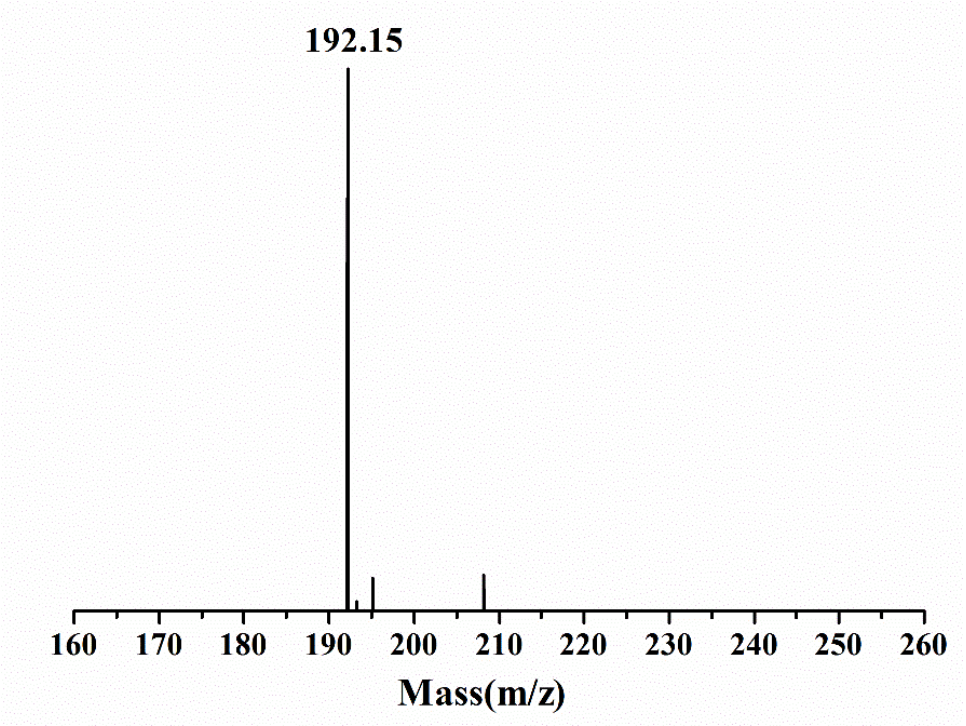


Fig. S2. MALDI-TOF Mass spectrum for methyl 4-((dimethylamino)methyl) benzoate.

Synthesis of 4-((dimethylamino)methyl)benzyl alcohol

To a solution of 4-((dimethylamino)methyl) benzoate (3.40 g, 17.6 mmol) in THF (100 ml) was added 4 equiv. $LiAlH_4$ (2.65 g, 70.5 mmol), at 0 ^{o}C and the mixture was stirred

under the nitrogen atmosphere at room temperature overnight. Excess $LiAlH_4$ was removed by filtration and the reaction mixture was separated with saturated $NaHCO_3$ and ethyl ester. The aqueous layer was extracted with ethyl ester and the combined organic layer was washed with brine, and dried over Na_2SO_4, and then was collected under reduced pressure. The residue was purified by silica gel column chromatography with CH_2Cl_2/CH_3OH at 30:1 (v/v) to give the product (yield: 70%, **Fig. S3-S5**).

^1H NMR (δ, ppm, $CDCl_3$): 7.34 (m, 4H, benzene); 4,69 (s, 2H, -C**H**$_2$OH); 3.48 (s, 2H, -NC**H**$_2$); 2.28 (s, 6H, -N(C**H**$_3$)$_2$).

^{13}C NMR (δ, ppm, $CDCl_3$):139.2 (benzene); 136.9 (benzene); 128.4 (benzene); 126.1 (benzene); 64.2 (-N**C**H$_2$); 62.8 (-**C**H$_2$OH); 44.3 (-N(**C**H$_3$)$_2$)

MALDI-TOF Mass Spectrum (M/z): calcd. $C_{10}H_{15}NO$ ($M\text{-}H^+$), 164.12; found, 164.16.

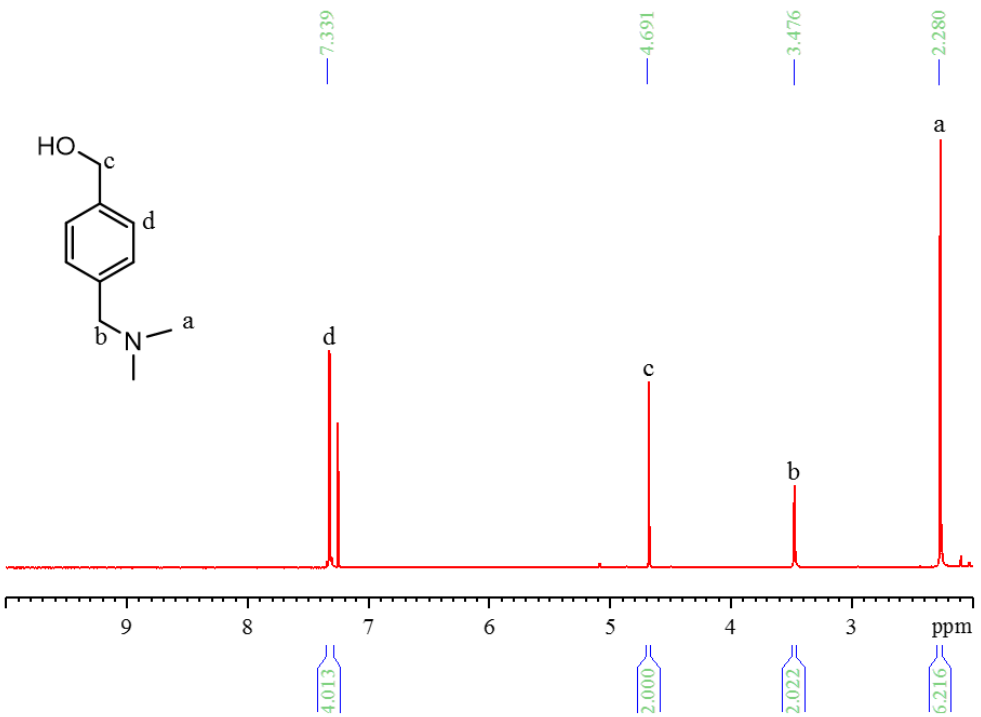


Fig. S3. ^1H NMR spectrum for 4-((dimethylamino)methyl)benzyl alcohol in $CDCl_3$.

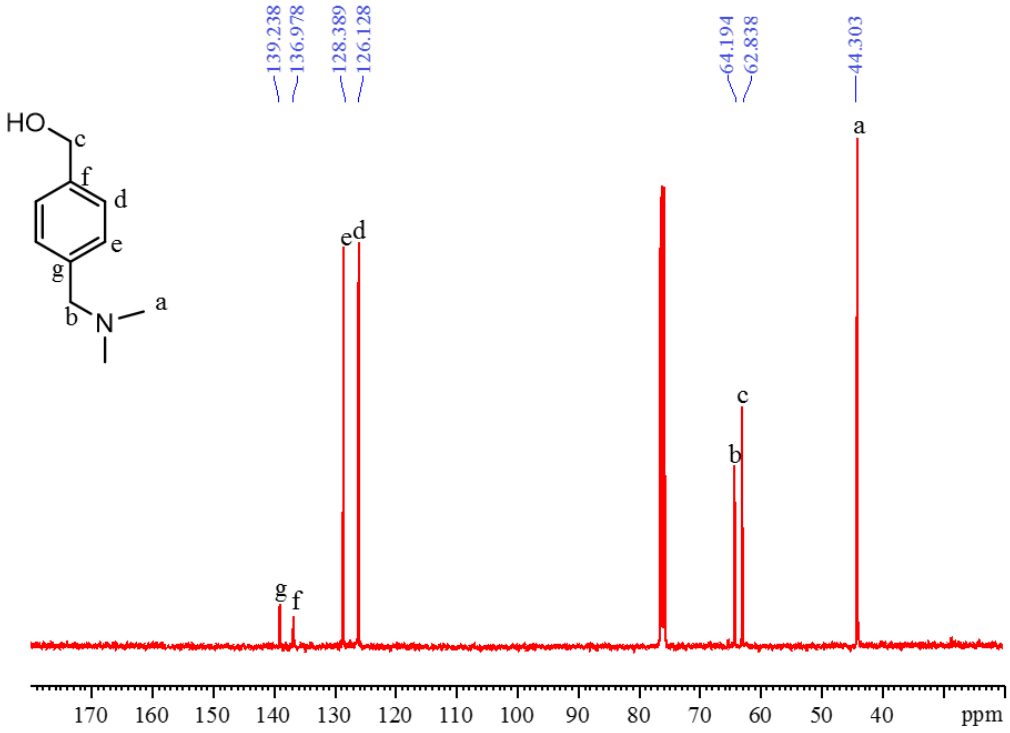


Fig. S4. ^{13}C NMR spectrum for 4-((dimethylamino)methyl)benzyl alcohol in $CDCl_3$.

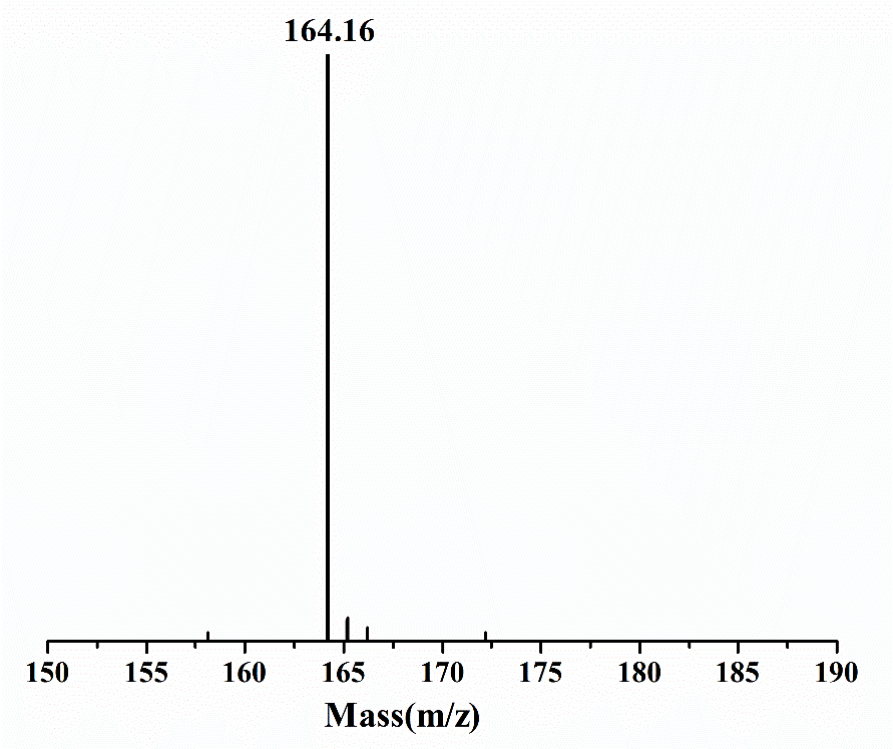


Fig. S5. MALDI-TOF Mass spectrum for 4-((dimethylamino)methyl)benzyl alcohol.

Synthesis of p-Dimethylamine methyl benzyl methacrylate ***(BMA)***.

To a solution of 4-((dimethylamino)methyl)benzyl alcohol (1.60 g, 9.7 mmol) and 3 equiv. ethyldiisopropylamine (DIPEA, 3.78 g, 29.0 mmol) in dry CH_2Cl_2, methacryloyl chloride (3.03 g, 29.0 mmol) in CH_2Cl_2 was added dropwise. The mixture was stirred at room temperature overnight. The reaction mixture treated with saturated sodium chloride and dried with Na_2SO_4, and followed by reduced pressure. The crude product was purified by column chromatography with CH_2Cl_2/CH_3OH at 30:1 (v/v) to obtain the product (yield: 75%, **Fig. S6-S8**).

^{1}H NMR (δ, ppm, $CDCl_3$): 7.33 (m, 4H, benzene); 6.15 (m, 1H, -C**H**$_2$); 5.58 (m, 1H, -C**H**$_2$); 5.18 (s, 1H, -OC**H**$_2$); 3.47 (s, 2H, -NC**H**$_2$); 2.28 (s, 6H, -N(C**H**$_3$)$_2$); 1.97 (s, 3H, -C**H**$_3$).

^{13}C NMR (δ, ppm, $CDCl_3$): 166.36 (-**C**OO-); 135.37 (benzene); 134.11 (benzene); 128.39 (benzene); 127.03 (benzene); 125.22 (-**C**H$_2$); 65.55 (-N**C**H$_2$); 63.29 (-**C**H$_2$OCO); 44.30 (-N(**C**H$_3$)$_2$); 17.41 (-**C**H$_3$).

MALDI-TOF Mass Spectrum (m/z): calcd. $C_{10}H_{15}NO$ ($M\text{-}H^+$), 232.14; found, 232.14.

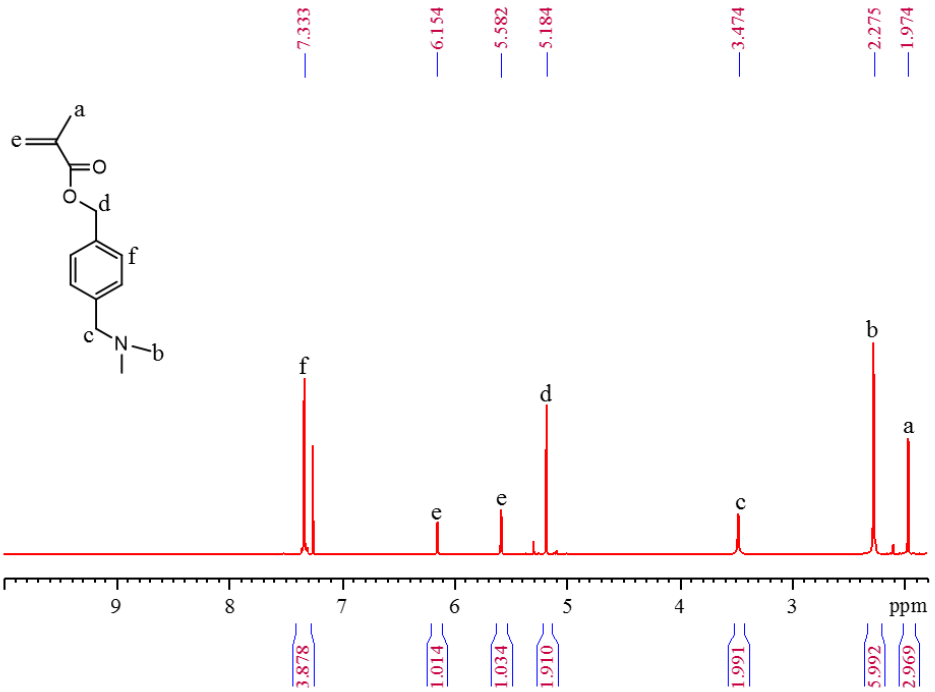


Fig. S6. ^{1}H NMR spectrum for 4-((dimethylamino)methyl) benzyl methacrylate in $CDCl_3$.

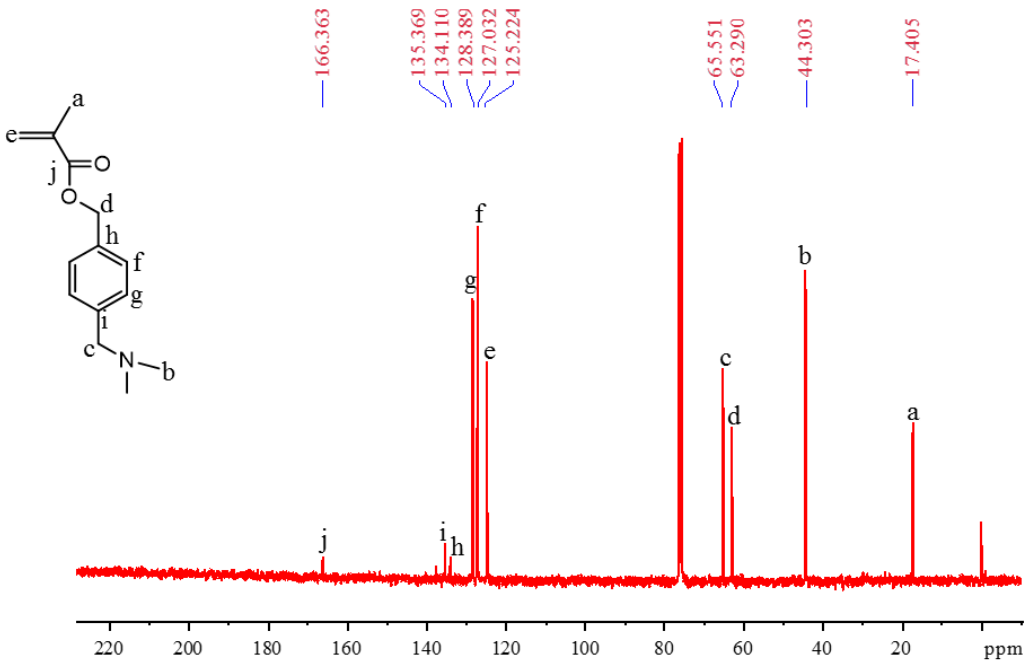


Fig. S7. ^{13}C NMR spectrum for 4-((dimethylamino)methyl) benzyl methacrylate in $CDCl_3$.

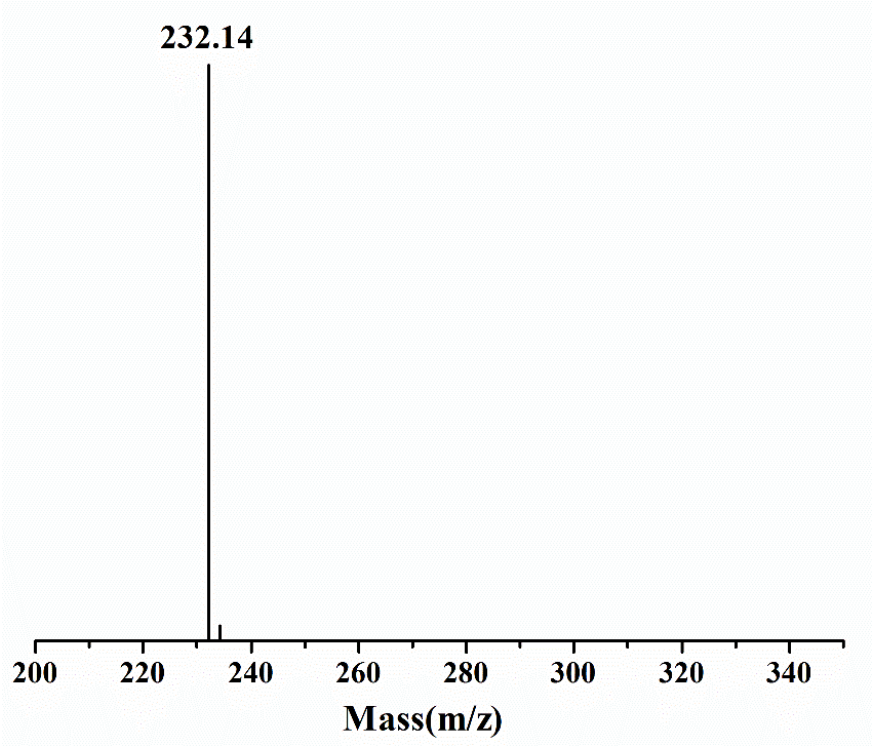
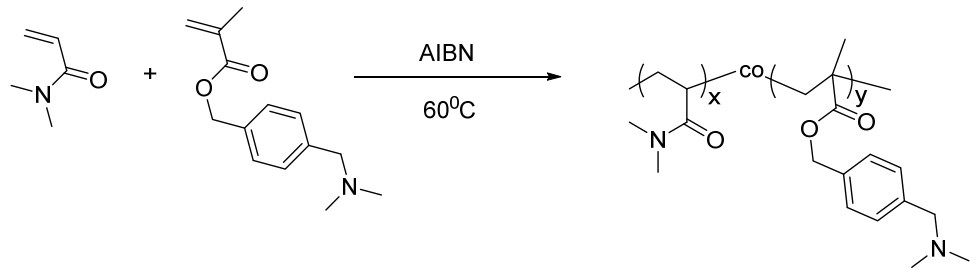


Fig. S8. MALDI-TOF spectrum for 4-((dimethylamino)methyl) benzyl methacrylate.

2.2 Synthesis of Water-Soluble Random Copolymers, Poly(dimethyl acrylamide)-co-Poly(p-Dimethylamine methyl benzyl methacrylate) via Free Radical Polymerization.



Scheme S2. Synthetic Route of Carbon Monoxide (CO)-Responsive Copolymer, PDMA-PBMA.

Dimethyl acrylamide (DMA), BMA, AIBN were dissolved in dry THF with the molar ratio of [DMA]:[BMA]:[AIBN] = *x*:*y*:0.01 ($x + y = 1$, $y = 0.02$, 0.05 and 0.10) and the mixture was stirred at 60 ^{o}C for 24 h under the N_2 atmosphere. The target polymer was purified by precipitation with diethyl ether. And the final polymer was $PDMA_x$-co-$PBMA_y$($x + y = 1$, $y = 0.01$, 0.03 and 0.07) respectively. The results were listed in the following **Table S1** and **Fig. S9–S12**.

^{1}H NMR (δ, ppm, CD_3CN): 7.31 (m, 4H, benzene); 4.94 (s, 2H, -OC**H**$_2$); 3.41 (s, 2H, -NC**H**$_2$); 2.87 (m, 347H, -N(C**H**$_3$)$_2$); 1.57(m, the proton at the polymer backbone).

Table S1. **The Polymerization Data of ($PDMA_x$-*co*-$PBMA_y$) copolymer.**

Entry	polymer structure	$M_{n,GPC}$ (Da)	M_w/M_n
1	$PDMA_{0.99}$-$PBMA_{0.01}$	12080	3.3
2	$PDMA_{0.97}$-$PBMA_{0.03}$	13000	2.8
3	$PDMA_{0.93}$-$PBMA_{0.07}$	12000	3.2

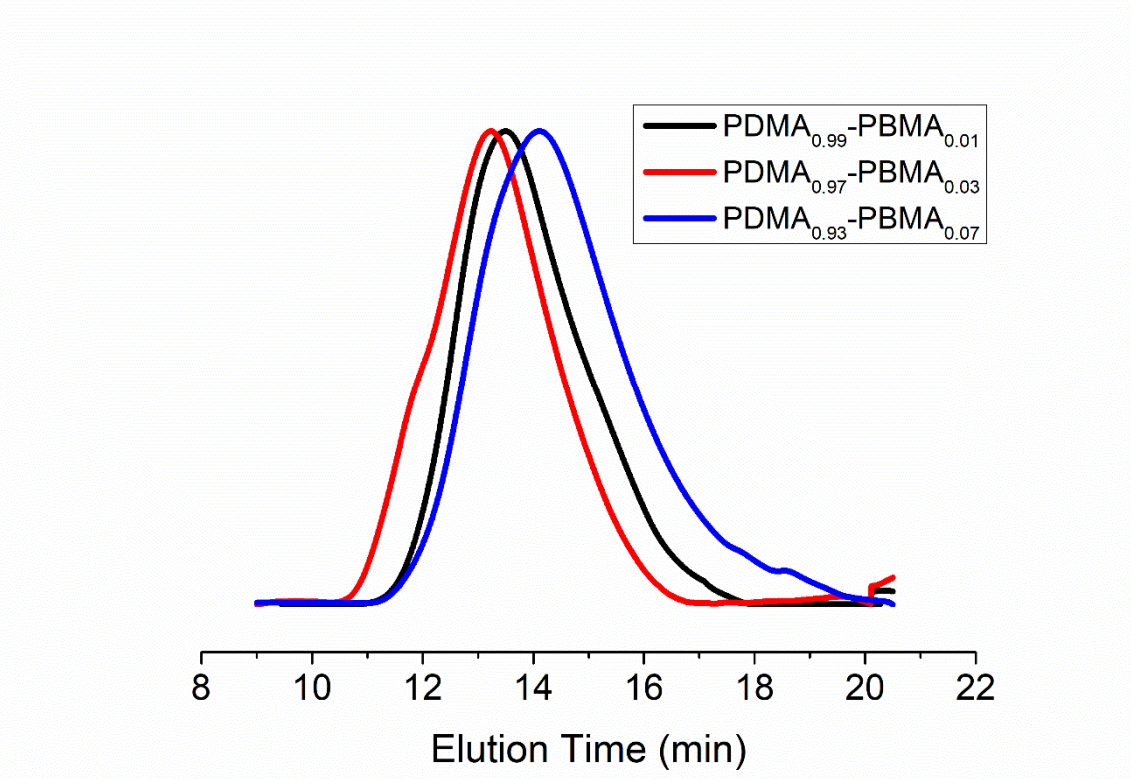


Fig. S9. GPC trace showing the molecular weight of the series of PDMA$_x$-PBMA$_y$ copolymers (y = 0.01, 0.03 and 0.07).

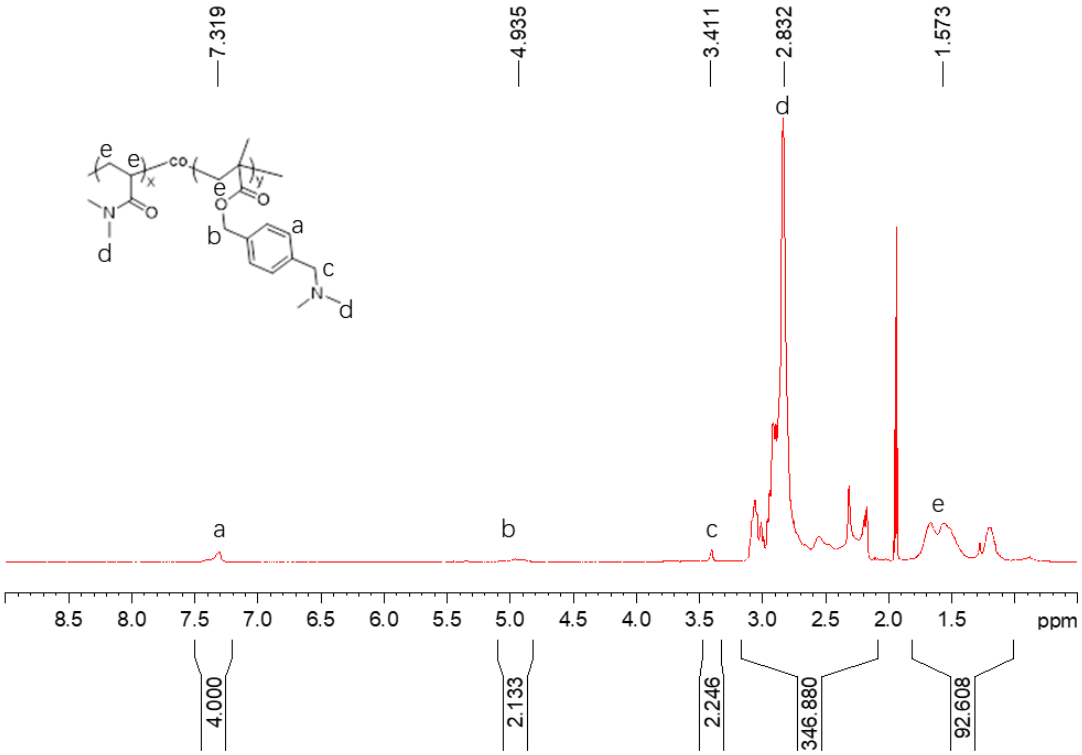


Fig. S10. ^{1}H NMR spectrum for PDMA$_{0.99}$-PBMA$_{0.01}$ copolymer in CD_3CN.

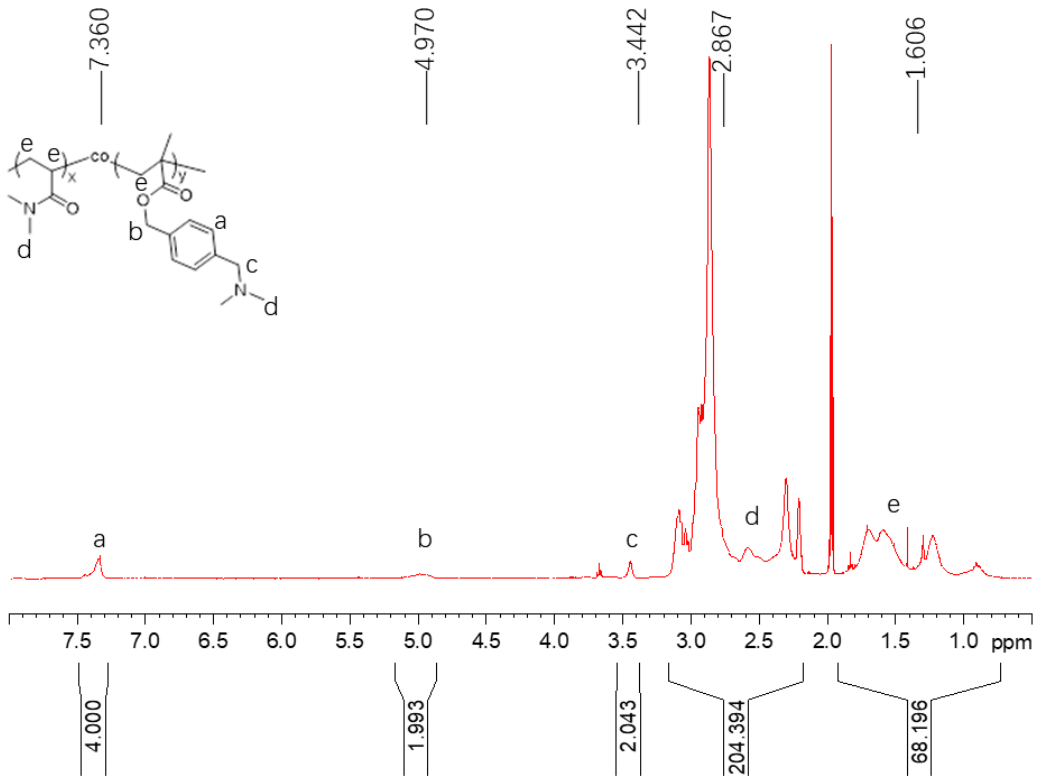


Fig. S11. ^{1}H NMR spectrum for $PDMA_{0.97}$-$PBMA_{0.03}$ copolymer in CD_3CN.

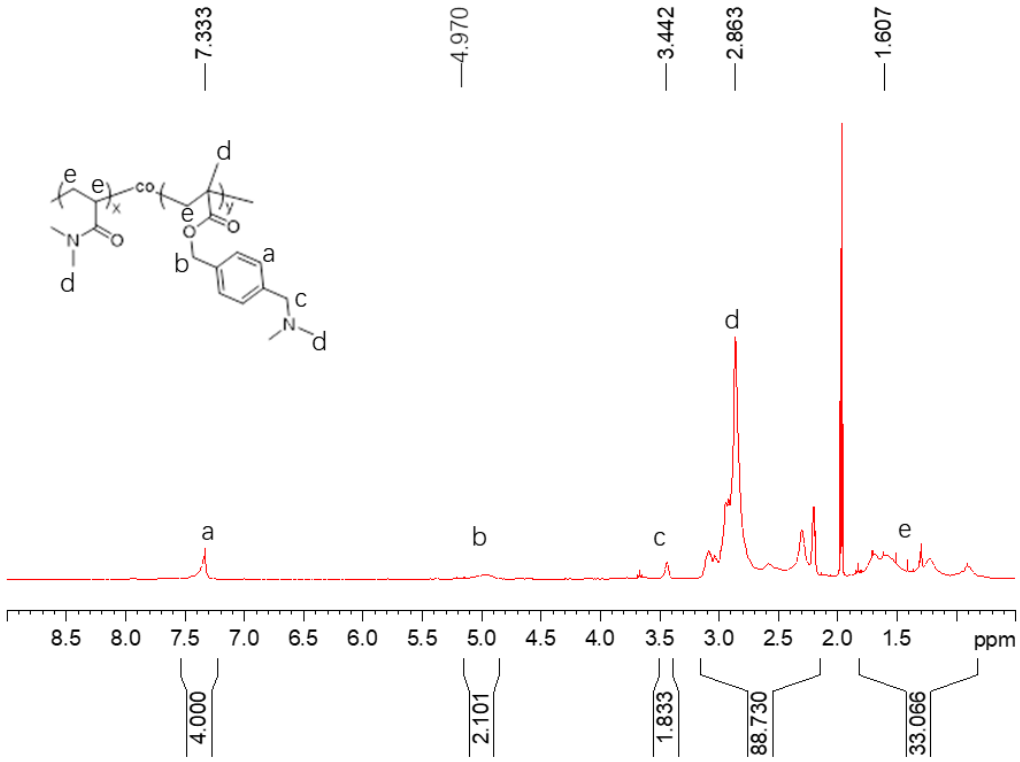


Fig. S12. ^{1}H NMR spectrum for $PDMA_{0.93}$-$PBMA_{0.07}$ copolymer in CD_3CN.

2.3 Preparation of CO and Other Similar Biological Irritants.

Carbon monoxide. CO irritant was used by an easy-to-handle CO releasing-molecule $Ru(CO)_3Cl$ (glycinate) (CORM-3) according to the literature.[1]

Nitric Oxide.1-Hydroxy-2-oxo-3-(N-methyl-3-aminopropyl)-3-methyltriazene (NOC-7) stock solution was prepared in 0.1 M NaOH. An appropriate amount of the stock solution was added to generate NO. The final mixtures were confirmed to have no notably pH change from 7.4.[2]

Peroxynitrite. $ONOO^-$ was prepared according to the literature and modified[3]. Briefly, to a solution of sodium hydroxide (30 mL, 1.5 M) was added sodium nitrite (30 mL, 0.6 M) and the mixture of hydrogen peroxide (30 mL, 0.7 M) and hydrochloric acid (0.6 M). It is noted that sodium nitrite and acidified hydrogen peroxide was added dropwise via a Y-type pipe with the same pump rate. Then activated manganese dioxide (4.0 g) was added to the resulted solution slowly to remove the excess hydrogen peroxide. After 15 min later the mixture was filtrated under reduced pressure and bright yellow filtrate was split into small aliquots and stored at lower than -18 °C. The operation of all of the above was carried out at 4 ºC. The concentration of the prepared peroxynitrite was determined by testing the absorption of the solution at 302 nm. The extinction coefficient of ONOO- solution in 0.1 M NaOH is 1670 M^{-1} cm^{-1} at 302 nm. C_{ONOO} = Abs302nm /1.67 (mM).

Hydrogen Sulfide. H_2S was obtained from the NaHS stock solution at pH = 4.5 acid environment.

Hydrogen Persulfide. We used Na_2S_2 as the hydrogen polysulfide donor and prepared a stock solution.

Hydrogen Peroxide. H_2O_2 (15 μM) was added to the polymer assemblies for selective responsiveness analysis.

Glutathione. GSH were used as received and prepared stock solution.

Hyperchlorite. 5% NaClO solution was purchased from Sigma. It was diluted with PBS to gain a stock solution. The pH was adjusted to 7.4 for adding to the hydrogel.

3. Palladation Reaction of the Copolymer and Formation of Pd-Bridged Polymer Hydrogels.

To a solution of copolymer $PDMA_{0.97}$-$PBMA_{0.03}$ (0.30 g) in 0.8 mL DMF, $Pd(OAc)_2$ (240 mg) dissolved in 0.2 mL benzene was injected slowly to make sure that the ratio of Pd to PBMA for all the samples is nearly 1:1. The mixture was stirred at 50 °C for 14 h under dark and dialyzed in water, an appearance change from a transparent solution to a black viscous gel suggested this Pd-induced gelation (**Fig. S13**). Other hydrogel for control listed in *Table 1* was obtained via the same method. Oscillatory frequency sweep experiments of these hydrogels by rheometer were exhibited in **Fig. S14**.

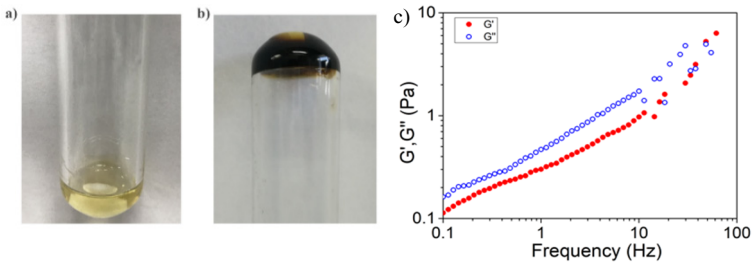


Fig. S13. Digital photographs showing an appearance change of $PDMA_{0.97}$-$PBMA_{0.03}$ copolymer from a transparent solution (a) to a black viscous gel (b) after Pd agent induced gelation via palladation reaction. c)The rheology data of the transparent solution without dynamic crosslinks.

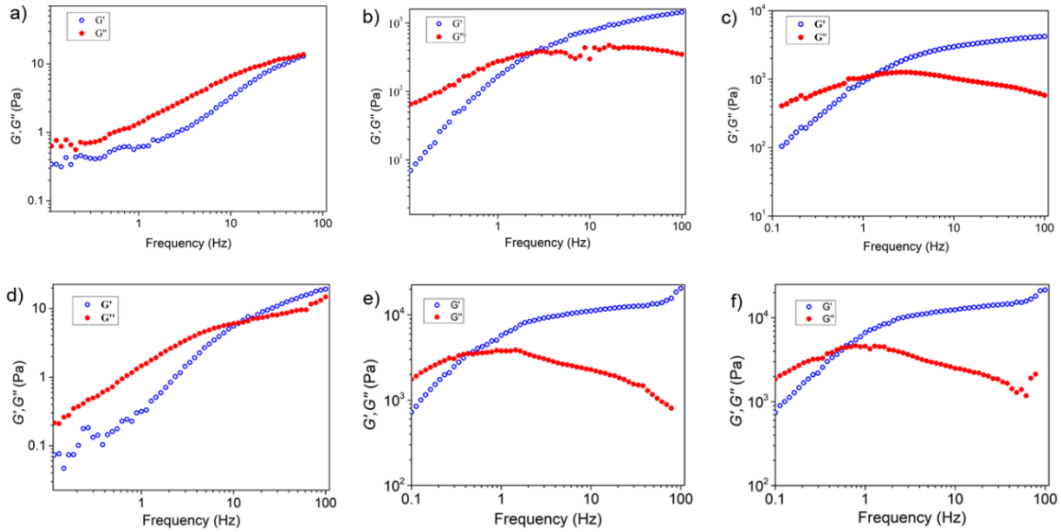
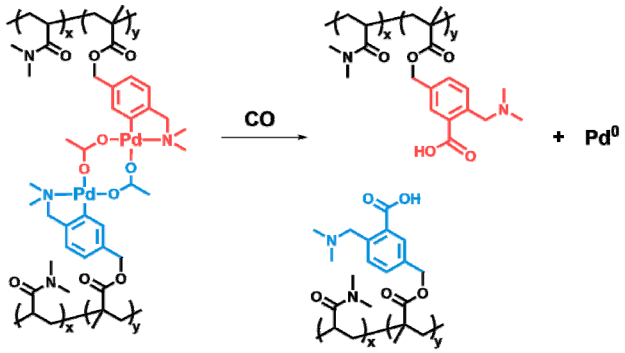


Fig. S14. Oscillatory frequency sweep experiments of Pd-bridged polymer samples. (a)-(c) $PDMA_{0.97}$-$PBMA_{0.03}$ polymer samples in 10%, 20% and 30% concentration, and (d)-(f) $PDMA_{0.93}$-$PBMA_{0.07}$ polymer samples in 10%, 20% and 30% concentration.

4. CO-Responsive Cleavage of Pd-Bridged Network



To elucidate the cleavage mechanism by CO, we analyzed the structure of the hydrogel before and after the introduction of CO by ^{1}H NMR. We found that both the signal of aromatic hydrogen and methylene hydrogen have a shift to low-field, which could be attributed to the disconnection of the Pd-cross-linking caused by a CO insertion reaction (**Fig. S15**).

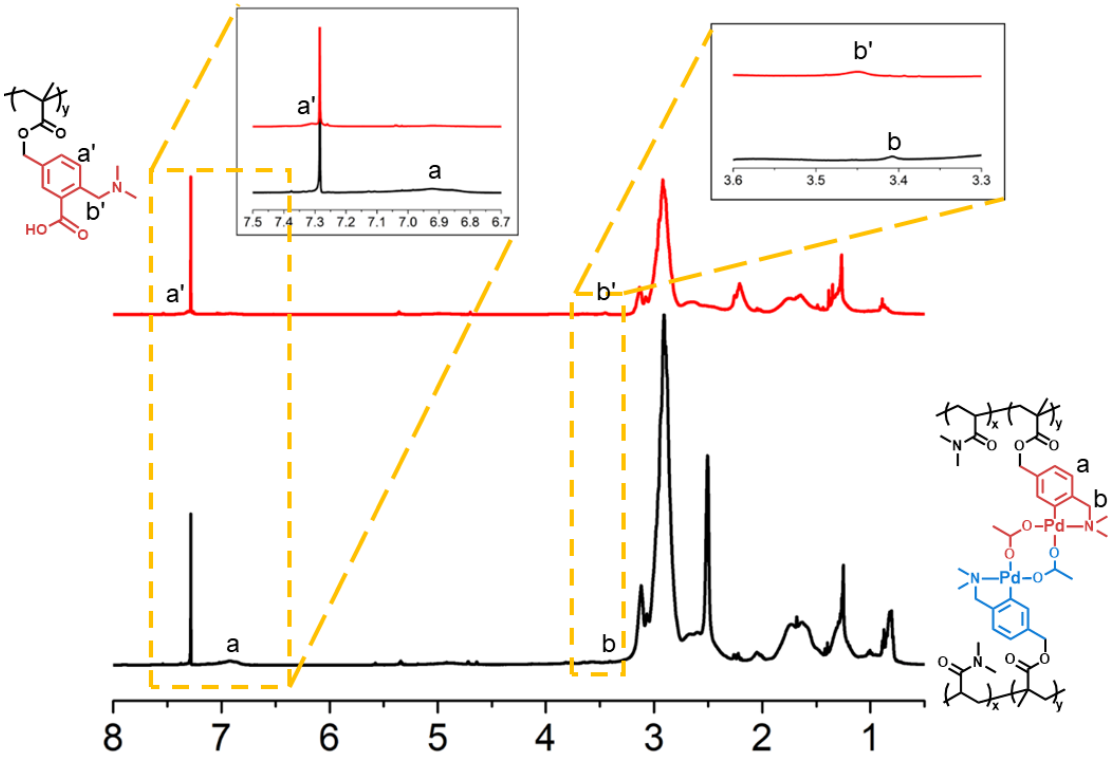


Fig. S15. Solid-state ^{1}H NMR spectra showing the cleavage mechanism of Pd-induced hydrogel by CO: Pd-bridged hydrogel (bottom panel) and CO-responsive cleavage of the Pd-bridged hydrogel (top panel). The inset images magnified the shifts in aromatic region and methylene peak.

5. Bio-selective Responsiveness toward CO Biosignal

After demonstrated that the Pd-bridged hydrogels possess CO-responsive dissociation ability, we expected that the hydrogel could be applied in physiological environment. Thus we want that the hydrogels should possess high bio-selectivity to only intracellular CO biosignal. However, in cells, besides CO, there are a panel of signaling molecules with analogous structure or function, such as NO, peroxynitrite ($ONOO^-$), H_2S, hydrogen persulfide (H_2S_2), glutathione (GSH), hydrogen peroxide (H_2O_2) and hypochlorite (OCl^-). To detect whether they have responsiveness, we used HAAKE Par Rotary Rheometer (HAAKE MARS III) to monitor their reactivity to the Pd-bridged hydrogel. As shown in **Fig. S16** and **Fig. S17**, except CO, other similar biological analytes have no ability to induce the hydrogel dissociation, which indicate that our hydrogels are of high-specific CO-responsiveness.

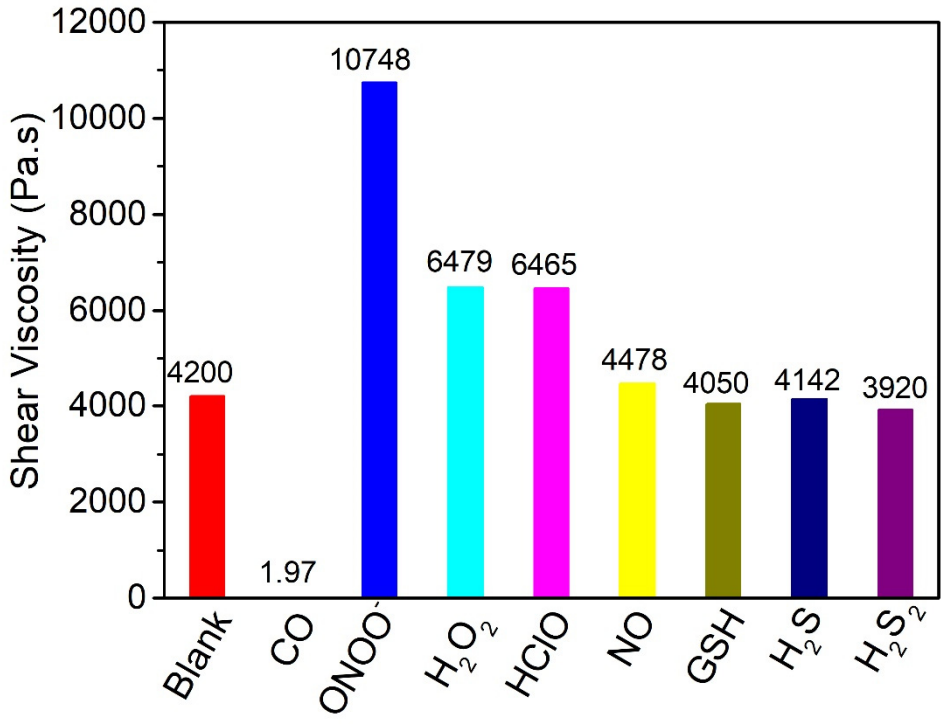


Fig. S16. The shear viscosity changes of hydrogels upon different biological signaling molecules.

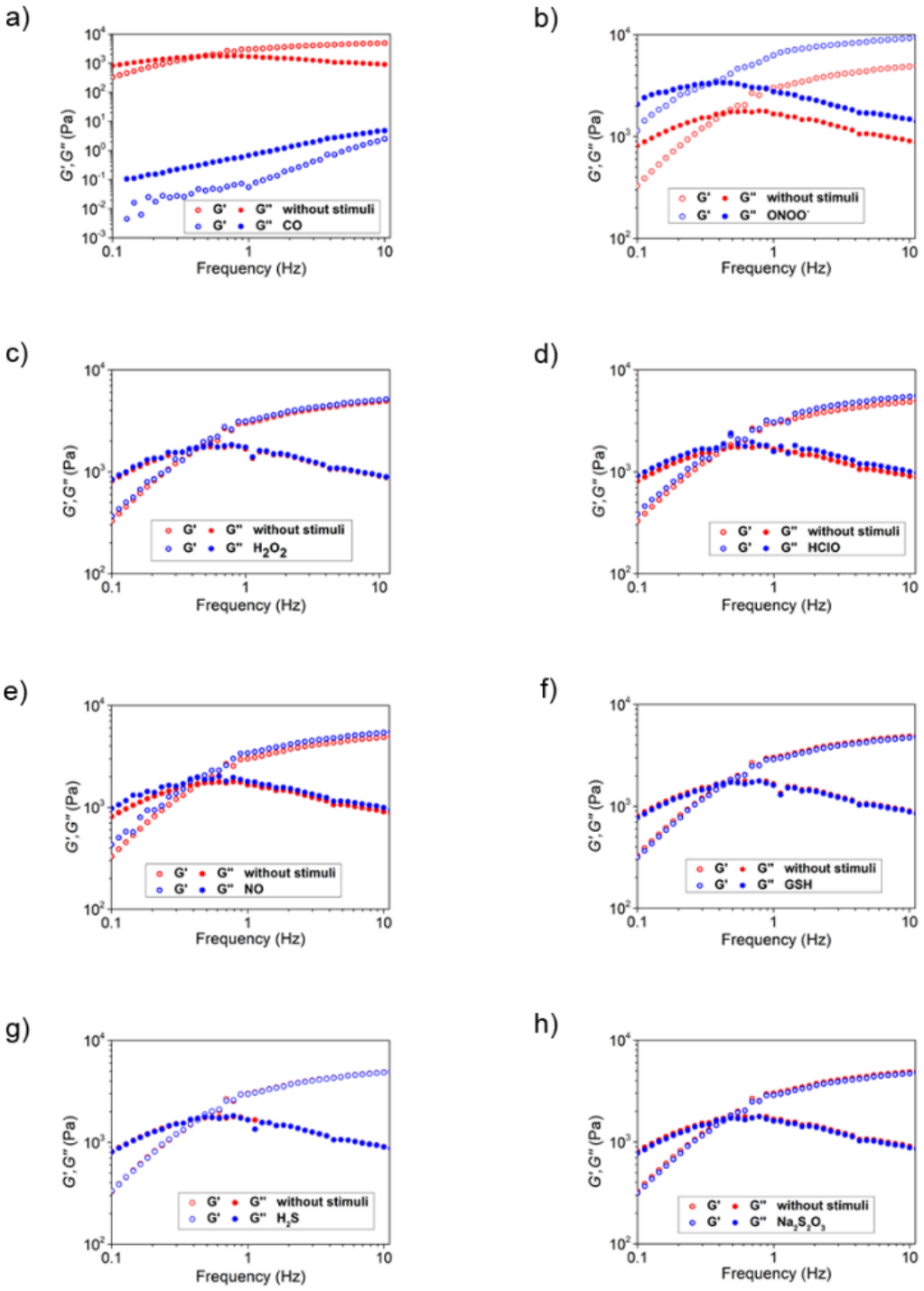


Fig. S17. The modulus changes of the Pd-bridged polymer hydrogels upon different biological analytes including: (a) CO, (b) $ONOO^-$, (c) H_2O_2, (d) HClO, (e) NO, (f) GSH, (g) H_2S, and (h) H_2S_2.

6. CO-Responsive Controlled Cargo Release from Pd-Bridged Polymer Hydrogels

The raw data of rhodamine B (RB) release amount was obtained with the aid of the RB standard fluorescent emission curve (λ_{em} = 564 nm) versus RB concentration. Every point in the standard curve was measured at a constant interval of half an hour.

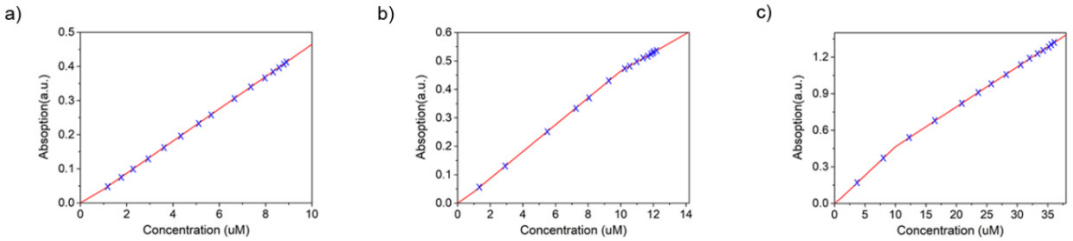


Fig. S18. The raw UV-Vis intensity for RB release under various CO levels: a) no stimulus, b) 0.1 equiv. CO to palladacycle unit, c) 0.3 equiv. CO to palladacycle unit.

7. Cargo Release Comparison with Other Similar Biological Analytes

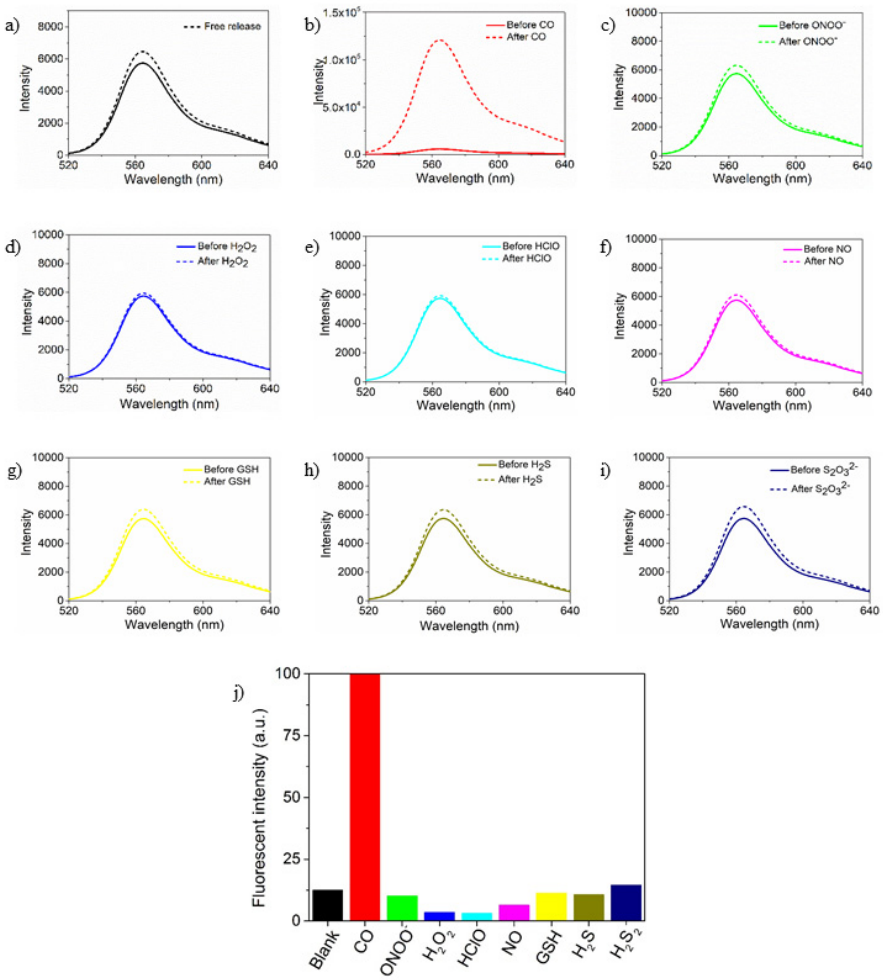


Fig. S19. Fluorescent intensity changes of the RB-loaded Pd-bridged hydrogels in different biological stimulus conditions. Only CO irritant showed a rapid and complete hydrogel dissociation, but other analytes only gave a low-level free-release. a) Blank. b) CO. c) $ONOO^-$. d) H_2O_2. e) HClO. f) NO. g) GSH. h) H_2S. i) H_2S_2. j) Bar graph of different biological stimulus.

Reference

(1) Clark, J. E.; Naughton, P.; Shurey, S.; Green, C. J.; Johnson, T. R.; Mann, B. E.; Foresti, R.; Motterlini, R. Circ. Res. 2003, 93, e2-e8.

(2) Johnson, I. D. The Molecular Probes Handbook: A Guide to Fluorescent Probes and Labeling Technologies; 11th ed.; Life Technologies Corporation, 2010.

(3) Reed, J. W.; Ho, H. H.; Jolly, W. L. J. Am. Chem. Soc. 1974, 96, 1248-1249.