Electronic Supplementary Material (ESI) for Biomaterials Science


# Supporting Information

# Hydrogels Formed by Oxo-ester Mediated Native Chemical Ligation

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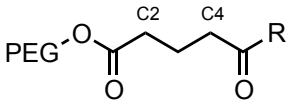
**Table S1.** $^{1}$H NMR chemical shift peak assignments used in calculation of relative abundance of species during reaction of P8NHS with L-Cys.




| Species | C2 | C4 |
|---|---|---|
| P8NHS | 2.61 | 2.86 |
| P8G | 2.52 | 2.47 |
| P8G-TE-Cys | 2.52 | 2.81 |
| P8G-AM-Cys | 2.52 | 2.43 |

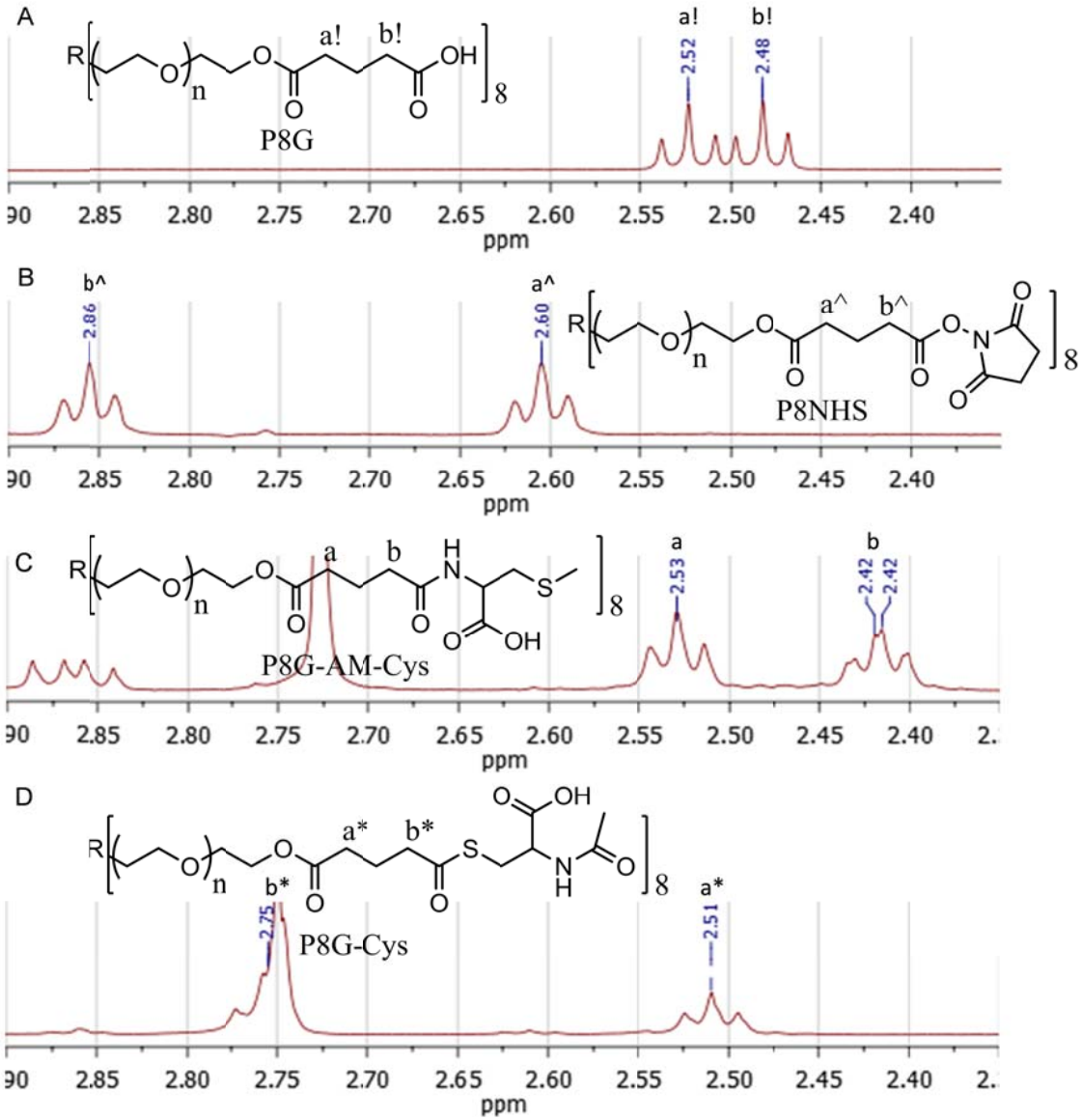



**Figure S1.** 1H NMR spectra and peak assignments for (A) P8G, (B) P8NHS, (C) P8G-TE-*N*-acetyl-Cys, and (D) P8G-AM-*S*-methyl-Cys in $D_2O$. The chemical shifts of the C2 and C4 protons of P8G-TE-*N*-acetyl-Cys and P8G-AM-*S*-methyl-Cys were taken as representative of P8G-TE-Cys and P8G-AM-Cys, respectively, in the calculation of relative abundance (RA) of species.

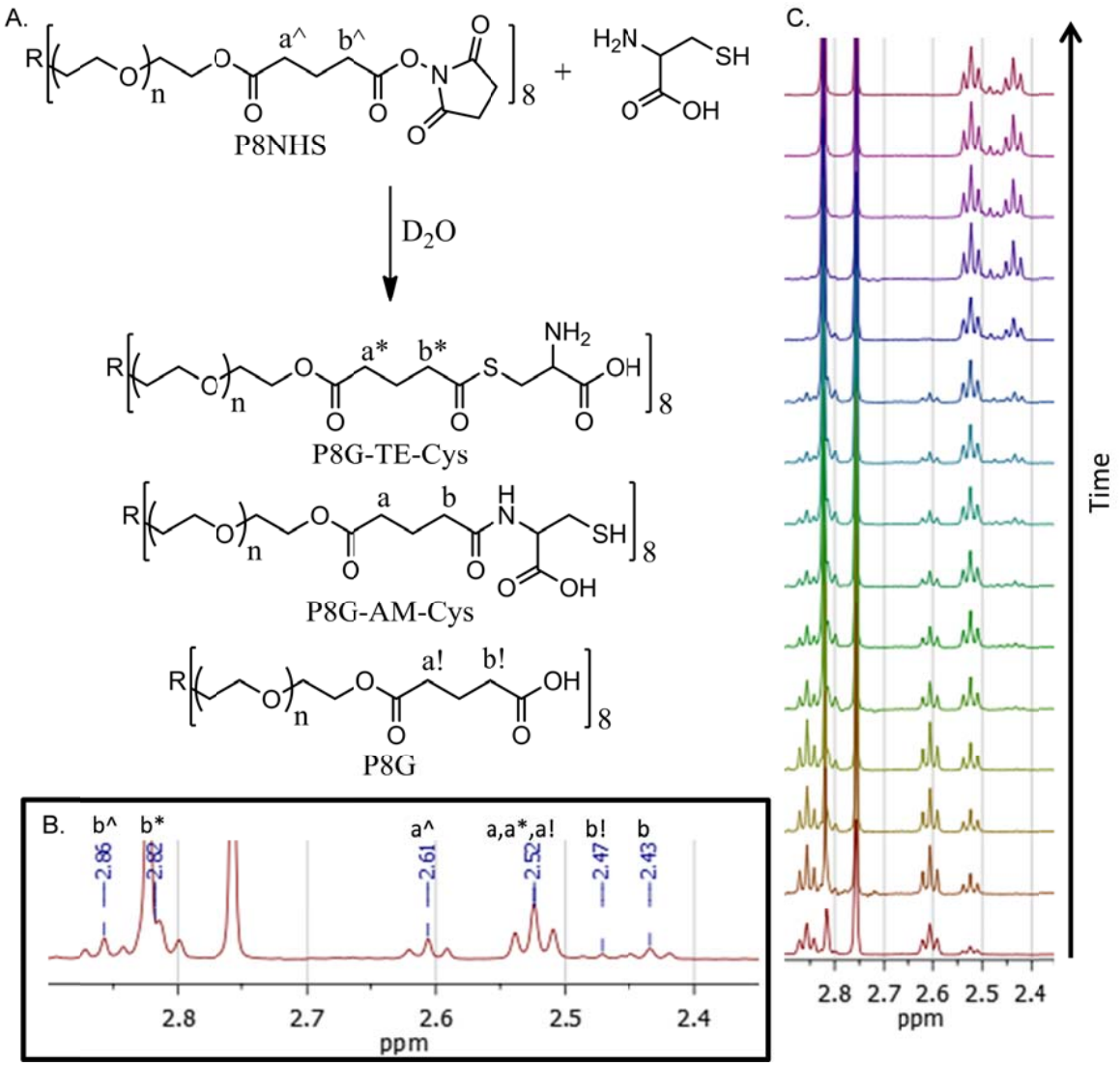



**Figure S2.** Kinetics of the reaction between P8NHS (1.25% (w/v), 5 mM NHS ester) and L-cysteine (29 mM) in unbuffered $D_2O$ (see main text for details). The two solutions were mixed in a 1:1 v/v ratio and the reaction followed by 1H NMR. (A) The four polymer species P8NHS, P8G-AM-Cys, P8G-TE-Cys and P8G were observed throughout the progress of the reaction. The protons on carbons C2 and C4 were used to quantify their relative abundances according to Equations 1-4 in the main text. (B) The proton chemical shifts were 2.52 ppm for a!, 2.47 ppm for b!, 2.86 ppm for b^, 2.61 ppm for a^, 2.52 ppm for a*, 2.82 ppm for b*, 2.52 ppm for a and 2.43 ppm for b. (C) The spectra shown correspond to reaction times ranging from 4.3 minutes to 121 hours. The disappearance of P8NHS peaks occurs as P8G-TE-Cys and P8G-AM-Cys peaks emerge.

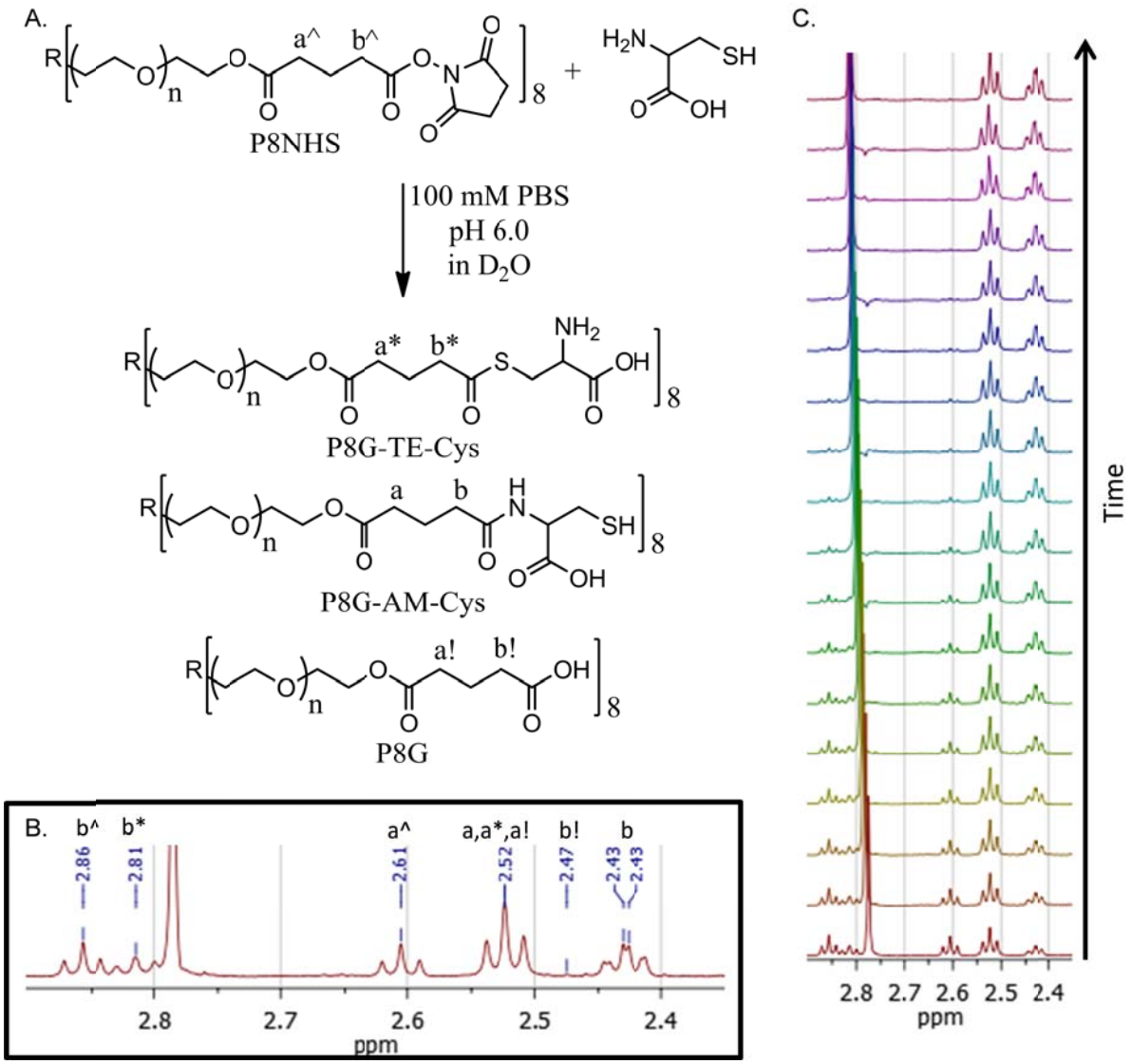



**Figure S3.** Kinetics of the reaction between P8NHS (10% (w/v), 38 mM NHS ester) and L-cysteine (57 mM) in buffered $D_2O$, pH 6.0 (see main text for details). The two solutions were mixed in a 1:1 v/v ratio and the reaction followed by 1H NMR. (A) The four polymer species P8NHS, P8G-AM-Cys, P8G-TE-Cys and P8G were observed throughout the progress of the reaction. The protons on carbons C2 and C4 were used to quantify their relative abundances according to Equations 1-4 in the main text. (B) The proton chemical shifts were 2.52 ppm for a!, 2.47 ppm for b!, 2.86 ppm for b^, 2.61 ppm for a^, 2.52 ppm for a*, 2.81 ppm for b*, 2.52 ppm for a and 2.43 ppm for b. (C) The spectra shown correspond to reaction times ranging from 3.2 to 85 minutes. The disappearance of P8NHS peaks occurs as P8G-TE-Cys and P8G-AM-Cys peaks emerge.

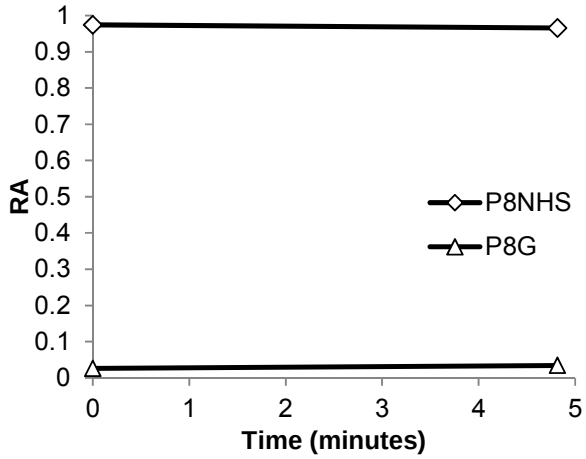



**Figure S4**. Kinetics of NHS hydrolysis for P8NHS in pH 7.0 buffer. Lyophilized 100 mM PBS (pH 7.0) was re-dissolved in $D_2O$ and used as the solvent for NMR analysis at a concentration of 10% (w/v) P8NHS (38 mM NHS ester). The relative abundance of P8G remained below 0.1 during the first 5 minutes of the reaction.

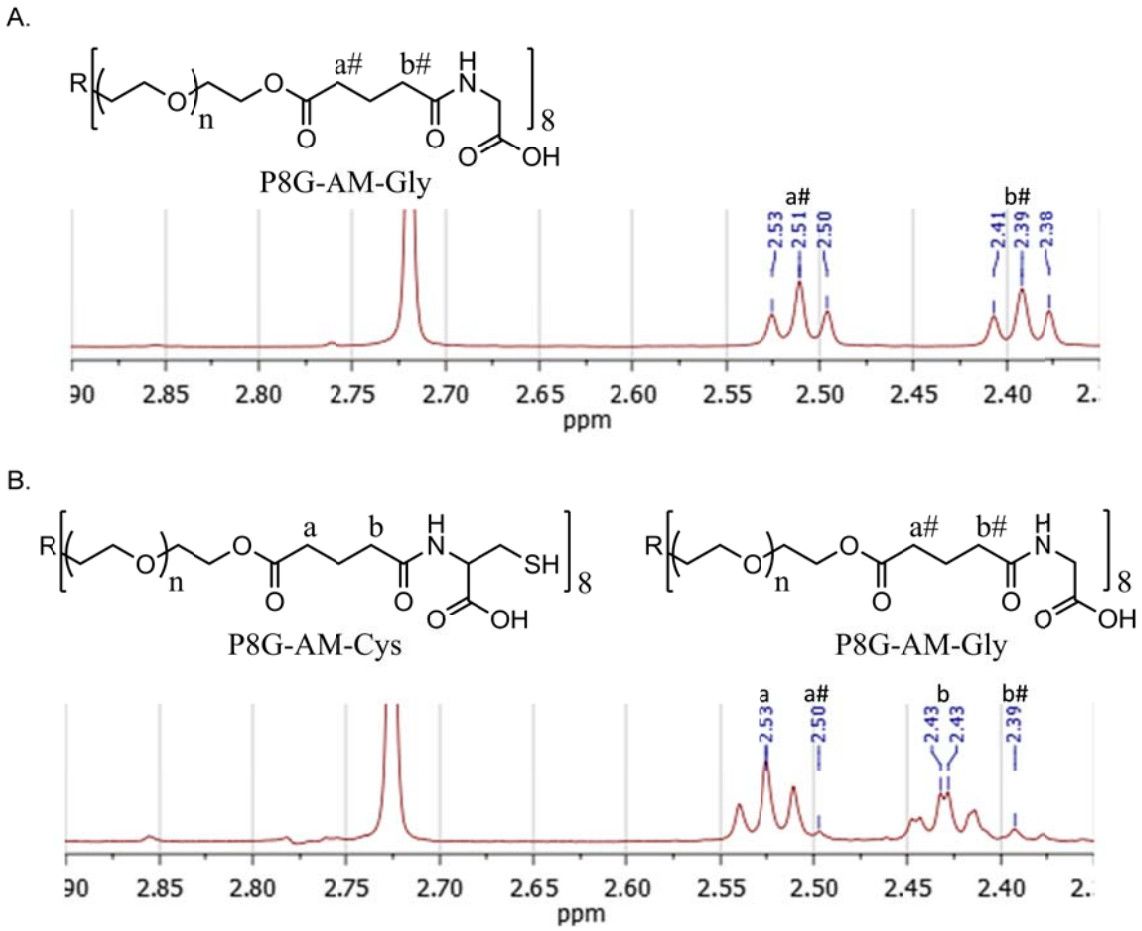



**Figure S5.** Reaction of P8NHS with L-Cys and L-Gly. (A) Peak assignment for C2 and C4 protons of P8G-AM-Gly in $D_2O$. (B) Reaction between P8NHS (10% (w/v), 38 mM NHS ester) and L-cysteine (57 mM) plus L-Gly (57 mM) in buffered $D_2O$, pH 7.0 (see main text for details). The two solutions were mixed in a 1:1 v/v ratio and the 1H NMR spectrum of the reaction mixture after 8 minutes is shown. Spectral analysis indicated that 80% of the product was P8G-AM-Cys, 15% was P8G-AM-Gly and 5% was P8G.

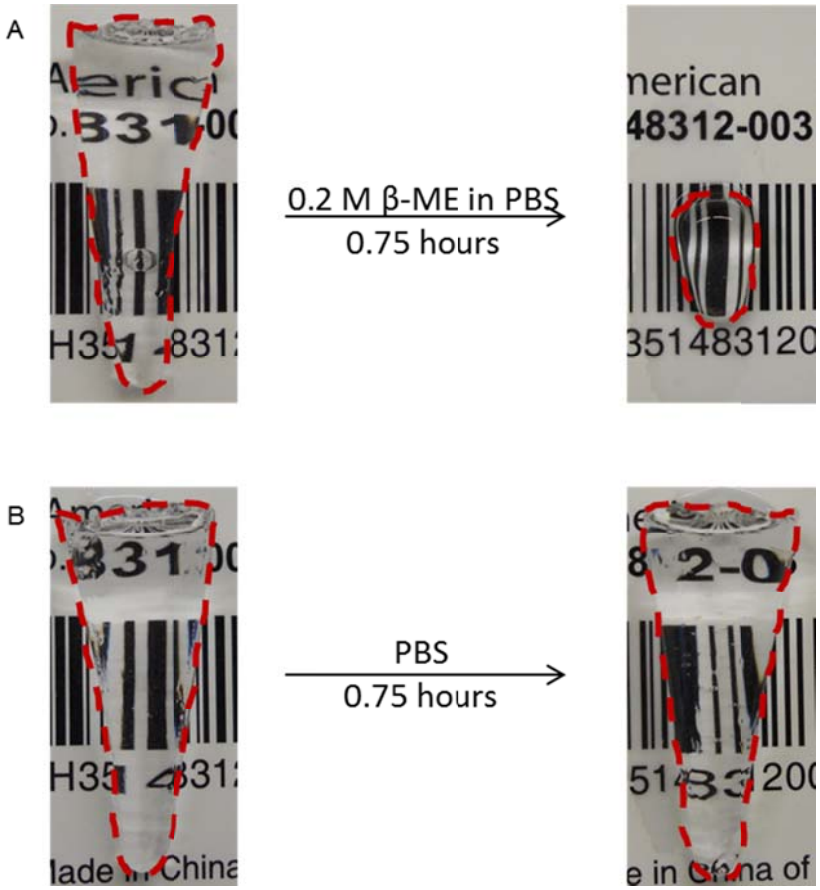



**Figure S6.** P8NHS plus L-Cys form hydrogels. In a microcentrifuge tube, 19 mM L-Cys in 100 mM PBS (pH 7.0) was mixed in a 1:1 v/v ratio with 10% (w/v) P8NHS (38 mM NHS ester) in 100 mM PBS (pH 7.0), forming stable hydrogels within 2 hours. (A) Incubation of one such hydrogel for 0.75 hours in PBS containing 0.2 M β-mercaptoethanol resulted in partial solubilization of the gel, and after 1.25 hours the gel was completely solubilized. (B) Following incubation of another such hydrogel for 0.75 hours in pure PBS demonstrated that the hydrogel remained intact. The edges of the hydrogels are marked with dashed lines.

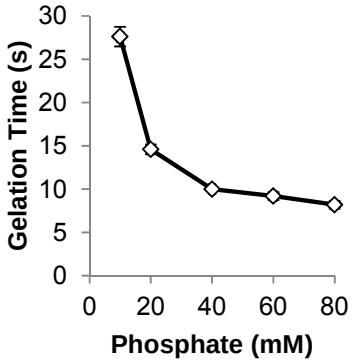



**Figure S7.** The effect of phosphate buffer concentration on gelation kinetics of 10% w/v hydrogels prepared in PBS (dilutions of 100 mM PBS) at room temperature with P8Cys and P8NHS (1:1 w/w). The plot shows gelation time versus phosphate buffer concentration for an initial buffer pH of 7.3.

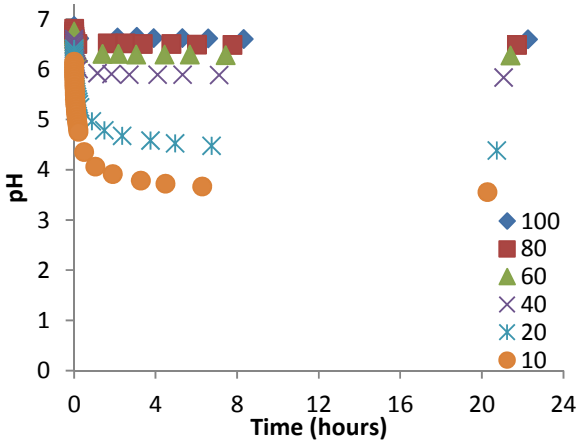



**Figure S8.** Change in pH during the reaction of P8NHS with L-Cys as a function of phosphate buffer concentration (10 to 80 mM, initial pH 7.3).

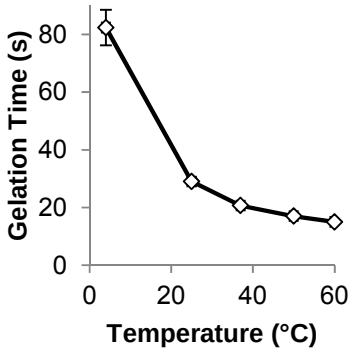



**Figure S9.** The effect of temperature on gelation time of 10% w/v hydrogels prepared with P8Cys and P8NHS (1:1 w/w) in 10 mM phosphate buffered saline (initial pH 7.0).

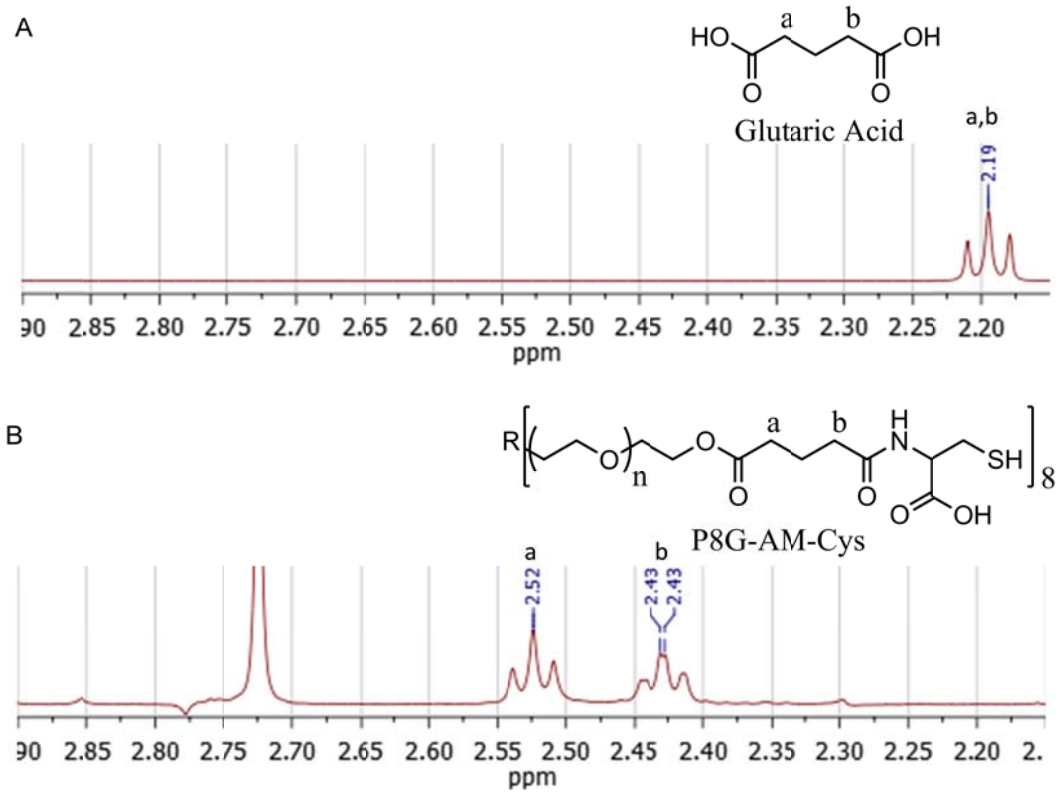



**Figure S10**. Analysis of potential hydrolysis for the ester between glutaric acid and PEG. Lyophilized 100 mM PBS (pH 7.0) was re-dissolved in $D_2O$ and used as the solvent for NMR analysis. (A) The 1H NMR spectrum of pure glutaric acid (5 mg/mL) showed that the protons located on carbons 2 and 4 of glutaric acid appear at 2.19 ppm. (B) 1H NMR spectrum for the reaction mixture of P8NHS and L-Cys at pH 7.0 at ~5 minutes (see method in the main text), revealing no appreciable hydrolysis of the ester between glutaric acid and PEG.

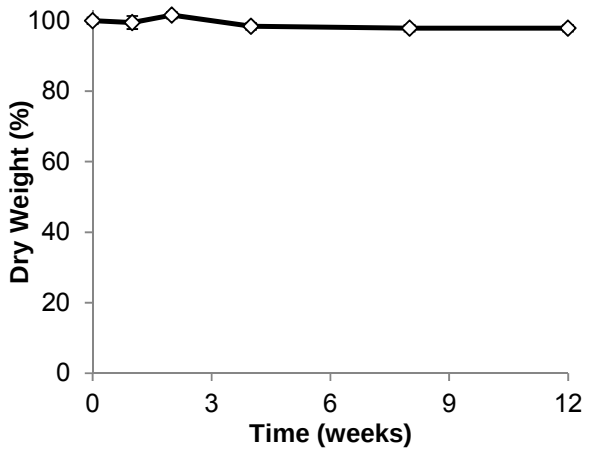



**Figure S11**. OMNCL hydrogel degradation in PBS. P8Cys and P8NHS were dissolved in PBS to yield 10% (w/v) solutions, mixed in a 1:1 (v/v) ratio, and allowed to gel for 15 minutes before being transferred into 2 mL of PBS. The 70 µL hydrogels were incubated at 37°C, and at various time points, 3 hydrogels were each washed with $ddH_2O$ before lyophilizing and dry weights measured. Following 12 weeks of incubation, no significant loss of dry weight was observed.