Supplementary Information

Tuning hydrolytic degradation of PEG-based thiol-norbornene hydrogels for multi-modal controlled release

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Supporting methods

Characterization of $PEGNB_{CA}$ or PEGNB thiol-norbornene hydrogels:

To obtain gel fractions of the different hydrogel formulations, PEGNB and $PEGNB_{CA}$ along with the thiol crosslinker were prepared in water when for PEG4SH and PEG8SH; however, crosslinking was unable to occur when the macromers were prepared in water for DTT. For this reason, we calculated the theoretical initial mass based upon the polymer wt% of the hydrogel when crosslinked with DTT. After crosslinking, the hydrogels were dried en vacuo for at least one day. Once dried, the mass of the hydrogel was measured to obtain (W_1). The hydrogels were then swelled in DDH_2O overnight to remove unreacted macromer from the hydrogel system. The hydrogels were then dried again and the mass was measured to obtain (W_2). Gel fraction was calculated from using the following equation.

$$Gel\ Fraction = \frac{W_2}{W_1} \tag{1}$$

Utilizing the mass swelling ratio as defined in the main paper, the average mesh size of thiol-norbornene click hydrogels were derived. The polymer volume fraction ($v_{2,s}$) was calculated using the following equation.

$$v_{2,s} = \frac{1}{Q} = \frac{{}^{1}\!/_{\rho_{H_2O}}}{\left[\left({}^{Q_m}\!/_{\rho_{PEG}}\right) + \left({}^{1}\!/_{\rho_{H_2O}}\right)\right]} \tag{2}$$

where Q is the volumetric swelling ratio. Since the hydrogel is in a highly swollen state, the volumetric swelling ratio can be estimated as the mass swelling ratio. ρ_{H_2O} is the density of water (0.994 g cm^{-3}) and ρ_{PEG} is the density of PEG (1.087 g cm^{-2}). From the calculated polymer volume

fraction, the number average molecular weight between crosslinks (M_C) was obtained using the Flory-Rehner equation.

$$\frac{1}{M_C} = \frac{2}{M_n} - \frac{\left(\bar{v}/V_1\right)\left[\ln\left(1 - v_{2,s}\right) + v_{2,s} + \chi_{1,2}{v_{2,s}}^2\right]}{\left({v_{2,s}}^{1/3} - \left(2/\phi\right)v_{2,s}\right)} \quad (3)$$

where M_n is the number average of molecular weight of polymer, $\chi_{1,2}$ is the polymer-water interaction parameter (i.e., PEG in water = 0.426, V_1 is the molar volume solvent (i.e., water = 18.0 cm^3/mol), $\bar{v}$ is the specific volume of PEG (i.e., 0.83494 cm^3/g) [1], and ϕ is the functionality of the crosslinker (i.e., 2 for DTT, 4 for PEG4SH, and 8 for PEG8SH, PEGNB, or $PEGNB_{CA}$). After average molecular weight between crosslinks was obtained, the average mesh size (ξ) was calculated using the following equation.

$$\xi = {v_{2,s}}^{-1/3}\left(\bar{r}_0{}^2\right)^{1/2} \quad (4)$$

where $\left(\bar{r}_0{}^2\right)^{1/2}$ is the root-mean-squared end-to-end distance of network chains in the unperturbed state (solvent free) and is defined as:

$$\left(\bar{r}_0{}^2\right)^{1/2} = l\left(3\frac{M_C}{M_r}\right)^{1/2}{C_n}^{1/2} \quad (5)$$

where l is the bond length (i.e., 0.146 nm for PEG), M_r is the molecular weight of polymer repeating unit (i.e., 44 g/mol for PEG), C_n is the characteristic ratio for the polymer (i.e., 4 for PEG) [2].

Supporting figures

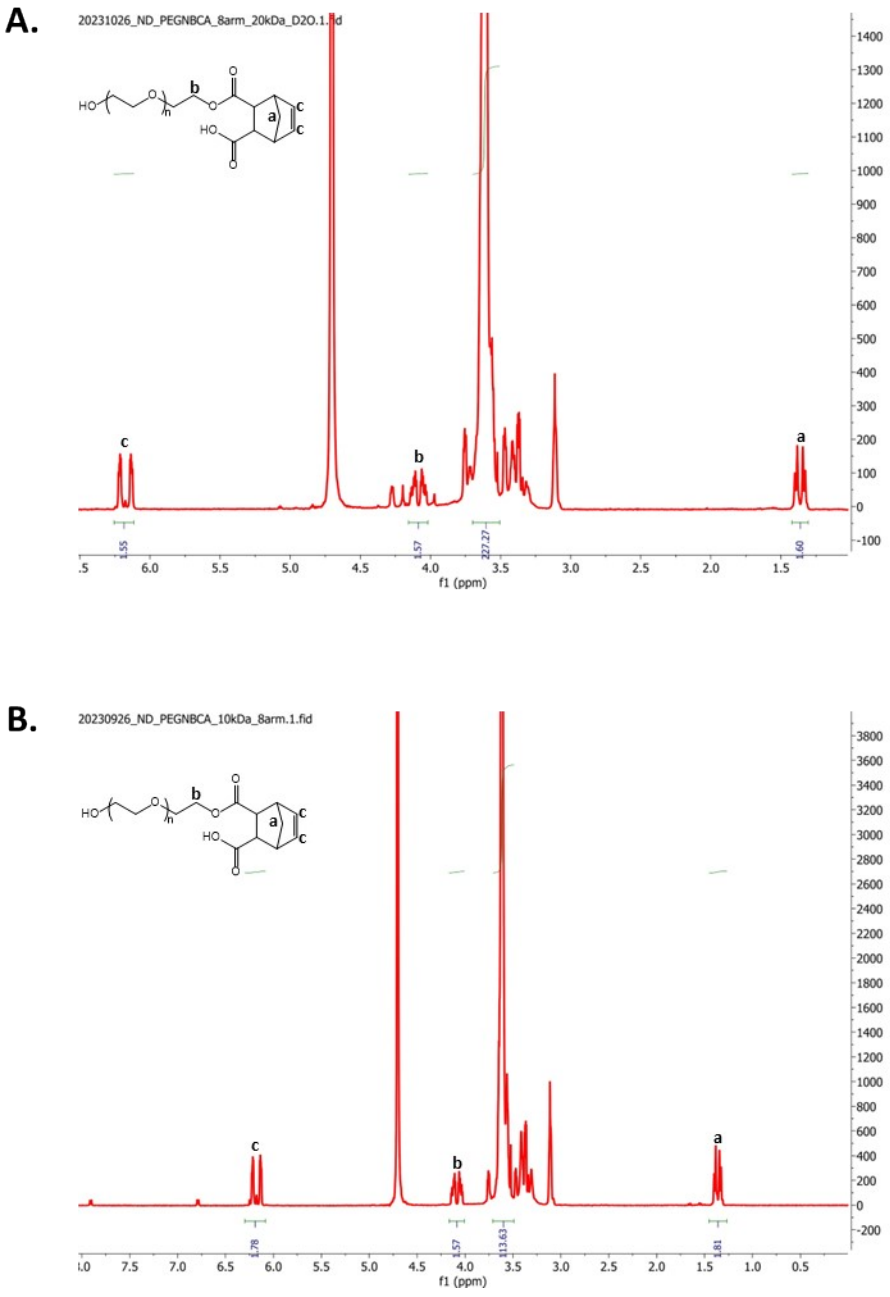

Figure S1. ^{1}H spectra of (A) $PEGNB_{CA}$ (8-arm, 10 kDa) and (B) $PEGNB_{CA}$ (8-arm, 20 kDa) in deuterium oxide using a Bruker Avance III 500 MHz NMR.

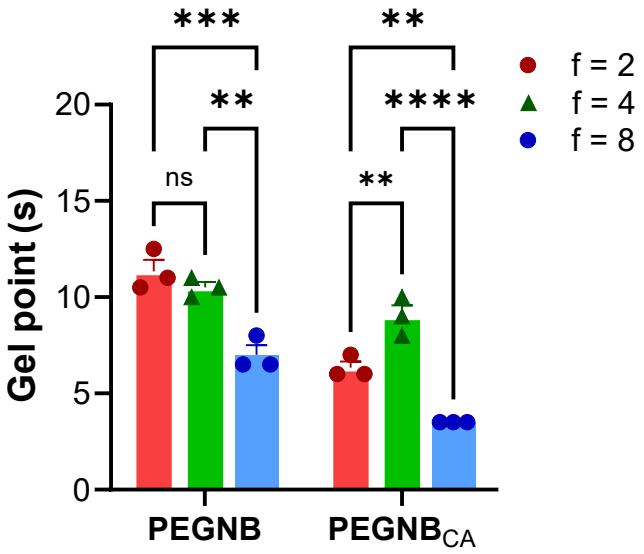

Figure S2. Gel points determined by *in situ* rheology of 5 wt% PEGNB or $PEGNB_{CA}$ (8-arm, 20 kDa) hydrogels crosslinked with thiol crosslinkers with different functionalities at R=1 with 2 mM LAP at 2 mW/cm^2 light intensity.

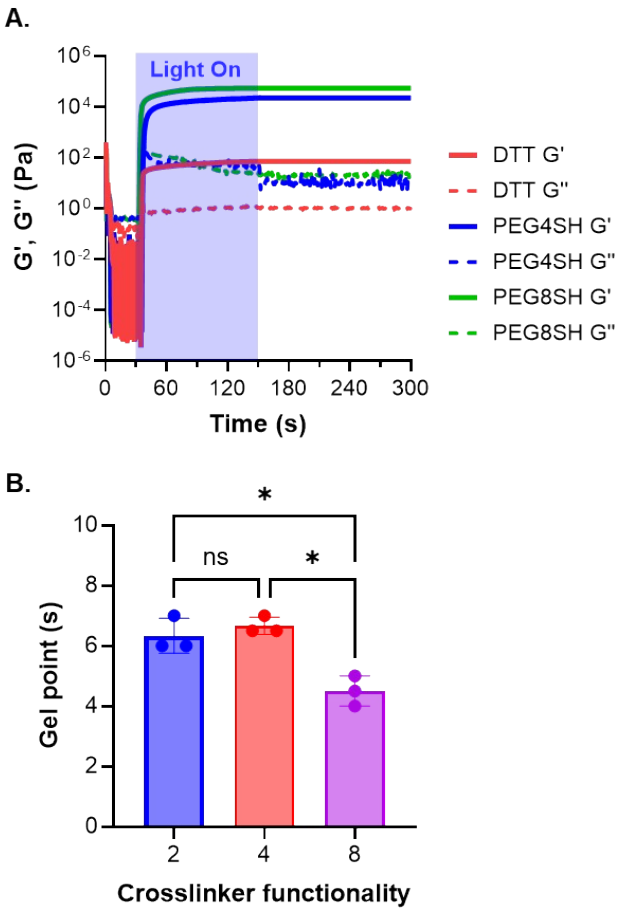

Figure S3. (A) *In situ* photorheometry of 5 wt% $PEGNB_{CA}$ (8-arm, 10 kDa) crosslinked with thiol crosslinkers with different functionalities at R=1 with 2 mM LAP at 2 mW/cm^2 light intensity. (B) Gel points identified from the *in situ* photorheometry plot.

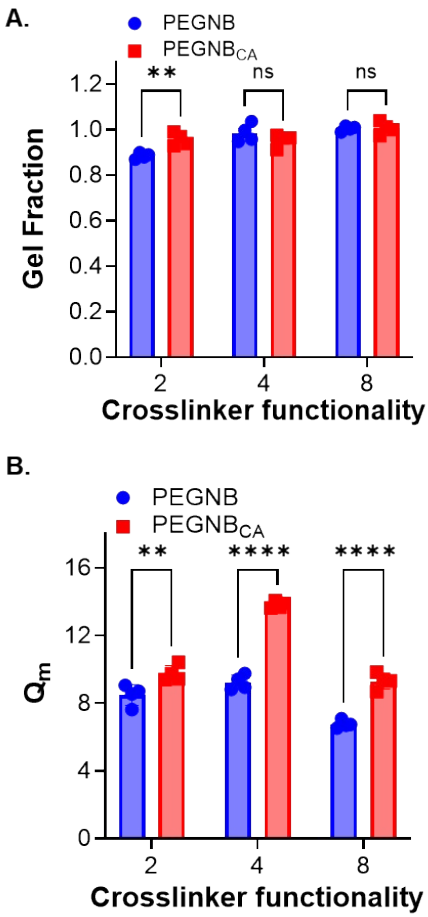

Figure S4. (A) Gel fraction or (B) mass swelling ratio of 10 wt% PEGNB or PEGNB$_{CA}$ (8-arm, 20 kDa) hydrogels crosslinked with PEG8SH at R=1.

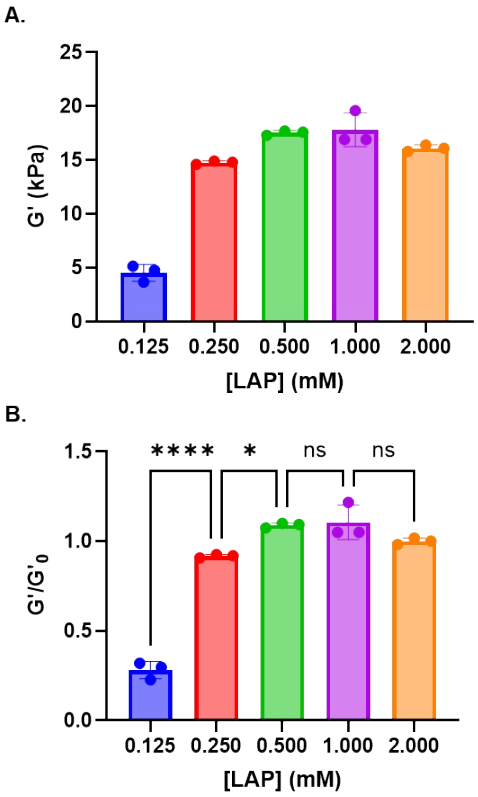

Figure S5. (A) Actual and (B) normalized elastic shear moduli of 5 wt% $PEGNB_{CA}$ (8-arm, 20 kDa) hydrogels crosslinked with PEG4SH at R=1. Values were normalized to the average G' of hydrogels crosslinked with 2 mM LAP.

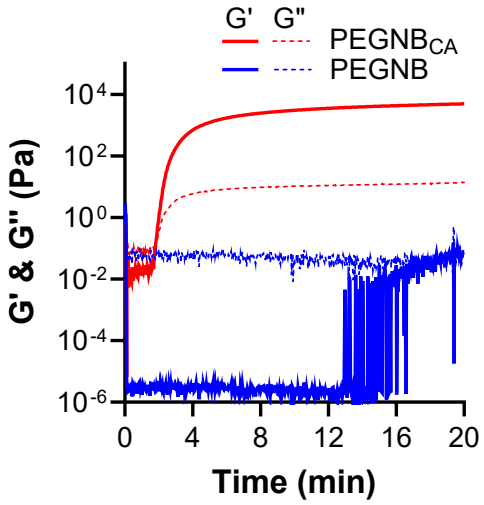

Figure S6. *In situ* rheometry of HRP-mediated crosslinking of 5 wt% PEGNB or PEGNB$_{CA}$ (8-arm, 20 kDa) with PEG8SH (R=1) and 0.5 kU/mL HRP in the absence of H_2O_2.

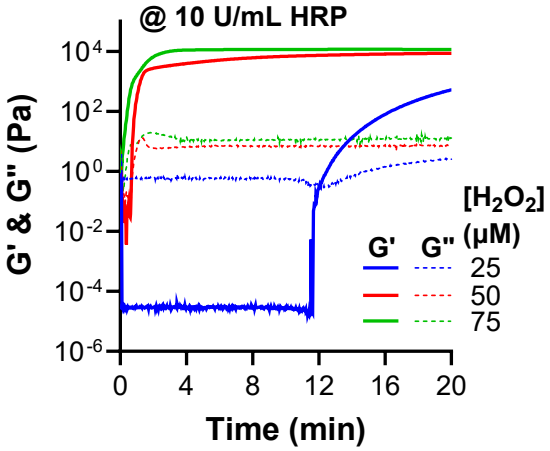

Figure S7. *In situ* rheometry of HRP-mediated crosslinking of 5 wt% $PEGNB_{CA}$ crosslinked with PEG8SH (R=1) and 10 U/mL HRP at different concentrations of H_2O_2.

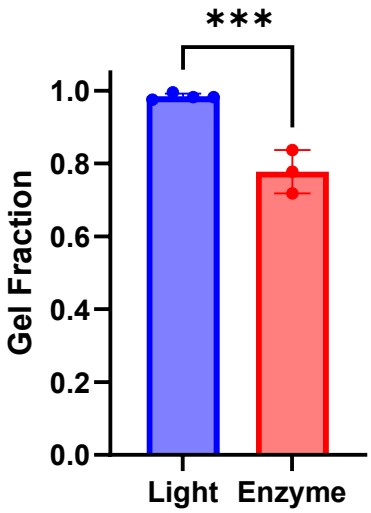

Figure S8. Gel fraction of 10 wt% $PEGNB_{CA}$ (8-arm, 20 kDa) hydrogels crosslinked with PEG8SH at R=1 through enzymatic (20 U/mL HRP) or light-mediated crosslinking (2 mM LAP, 20 mW/cm^2 at 365 nm).

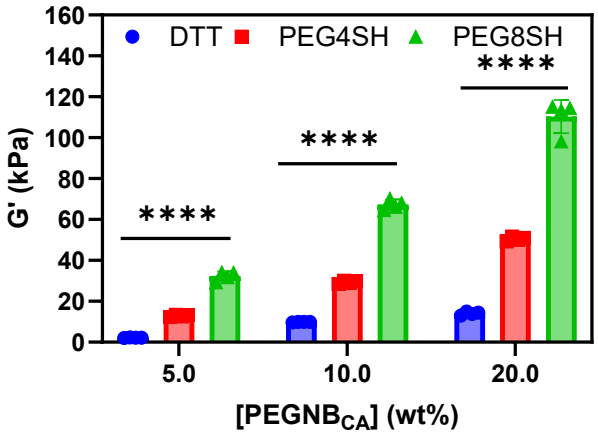

Figure S9. Initial elastic shear moduli of $PEGNB_{CA}$ (8-arm, 20 kDa) hydrogels at different macromer content crosslinked with different thiol crosslinkers at R=1.

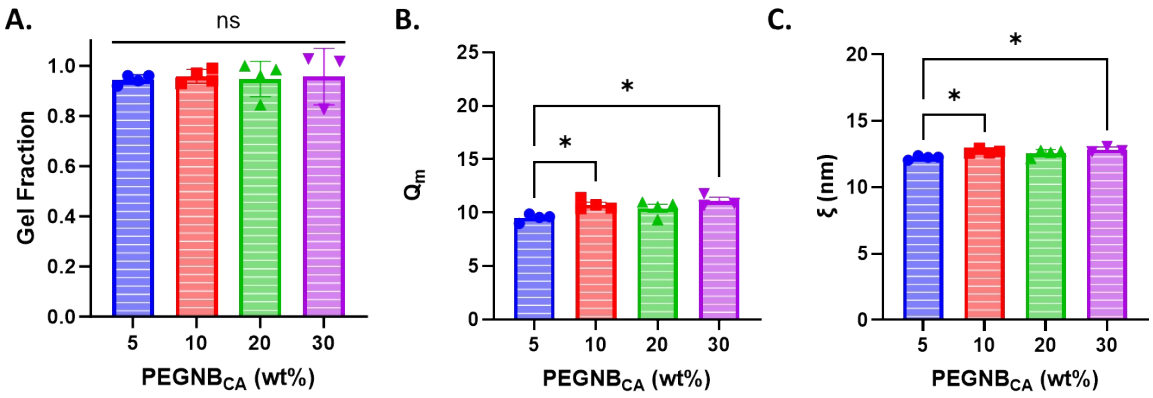

Figure S10. (A) Gel fraction, (B) mass swelling ratio, and (C) mesh size of $PEGNB_{CA}$ (8-arm, 20 kDa) hydrogels crosslinked by DTT.

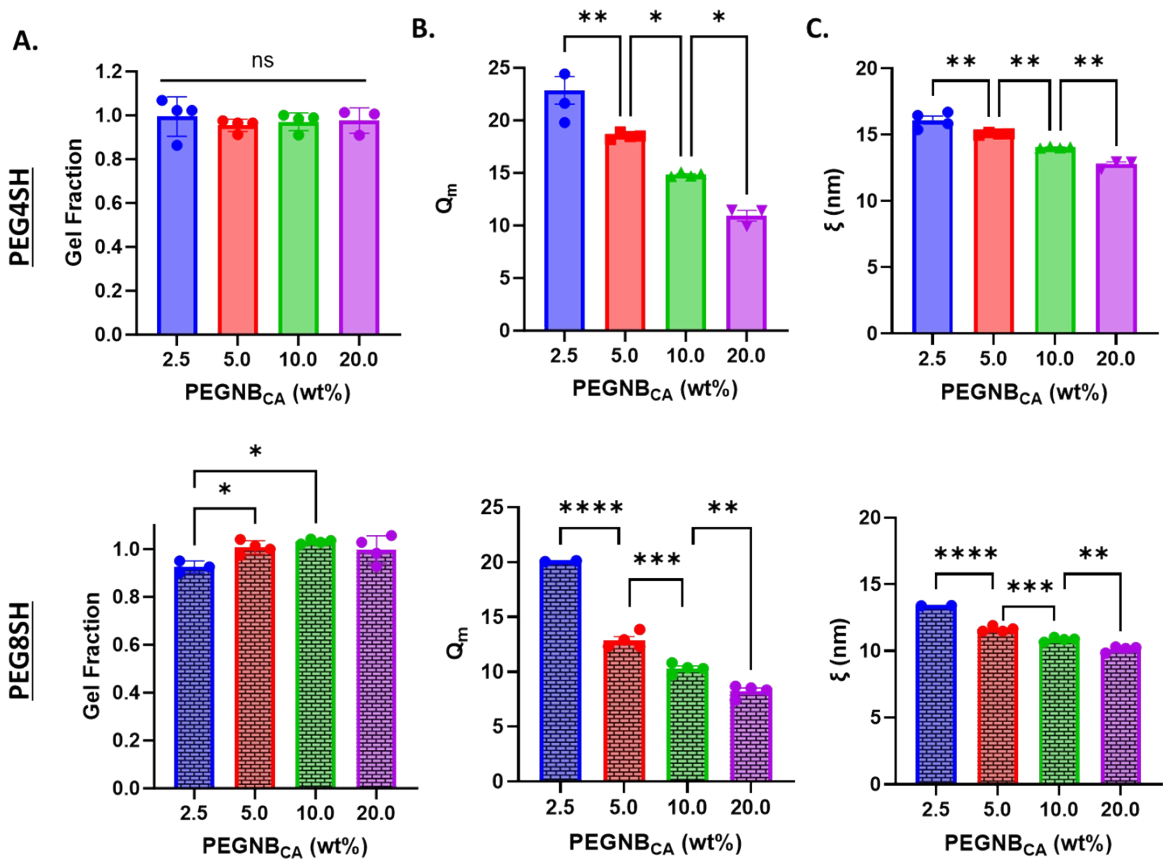

Figure S11. (A) Gel fraction, (B) mass swelling ratio, and (C) mesh size of PEGNB$_{CA}$ (8-arm, 20 kDa) hydrogels crosslinked by PEG4SH (top row) or PEG8SH (bottom row).

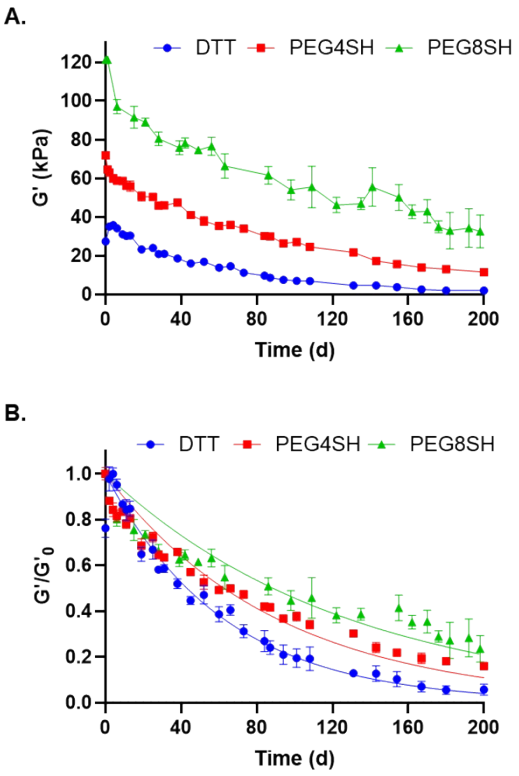

Figure S12. Changes in (A) elastic shear moduli and (B) normalized elastic shear moduli over time of 10 wt% PEGNB (8-arm, 20 kDa) hydrogels crosslinked with different thiol crosslinkers.

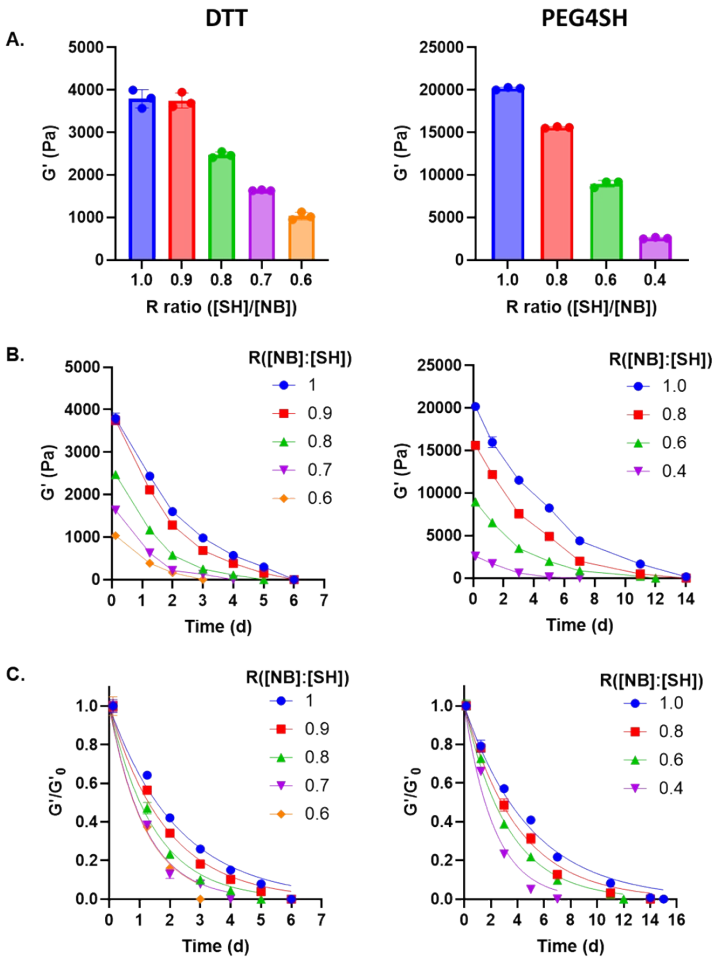

Figure S13. Effect of R ratio on hydrolytic degradation. (A-B) Initial elastic shear moduli of 5 wt% $PEGNB_{CA}$ (8-arm, 20 kDa) crosslinked with (A) DTT or (B) PEG4SH at different R ratios. (B-C) Changes in (B) actual or (C) normalized elastic shear moduli overtime for 5 wt% $PEGNB_{CA}$ (8-arm, 20 kDa) crosslinked with DTT or PEG4SH at different R ratios.

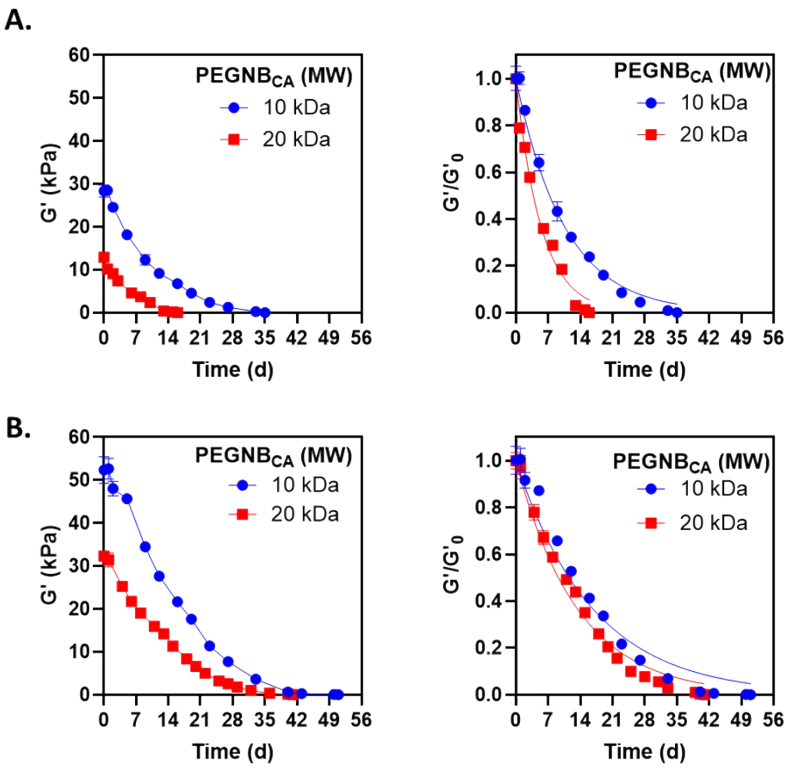

Figure S14. Effect of molecular weight of $PEGNB_{CA}$ on hydrolytic degradation. (A-B) Changes in elastic shear moduli and normalized elastic shear moduli over time of 5 wt% $PEGNB_{CA}$ (8-arm, 10 kDa or 20 kDa) crosslinked with (A) PEG4SH or (B) PEG8SH at R=1.

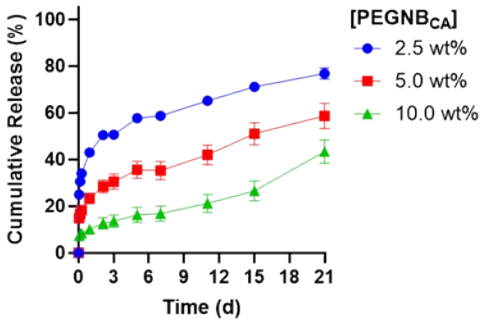

Figure S15. Release of 70 kDa FITC-Dextran from PEGNB$_{CA}$ (8-arm, 10 kDa) hydrogels crosslinked with PEG8SH at R=1.

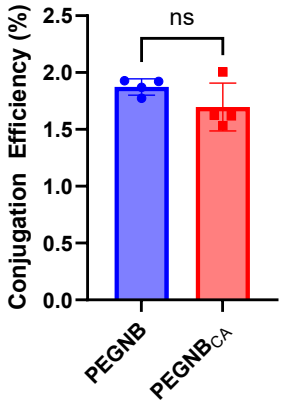

Figure S16. Conjugation efficiency of PEG-fluorescein-thiol (3.4 kDa) onto 10 wt% PEGNB or PEGNB$_{CA}$ crosslinked with PEG8SH at R=0.8.

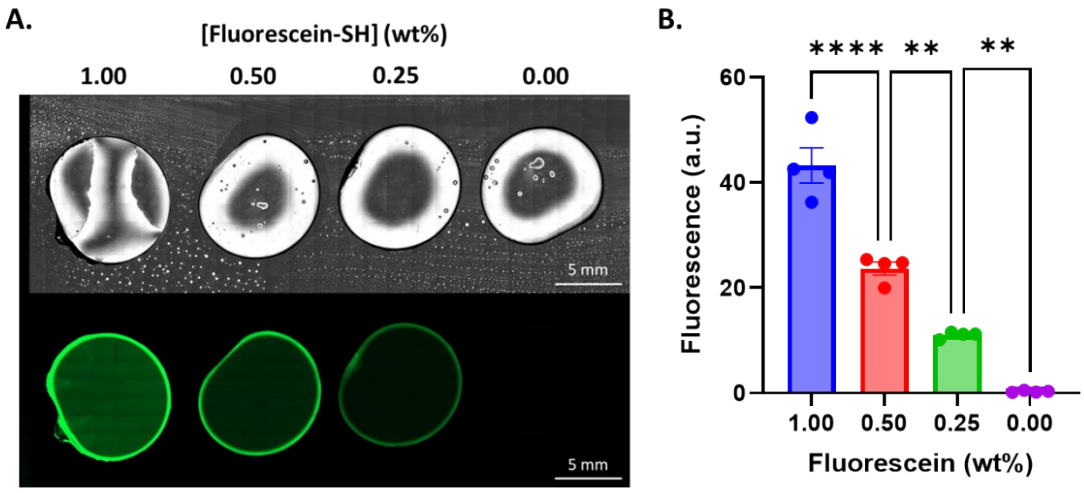

Figure S17. (A) Brightfield and fluorescent imaging (BioTek Lionheart microscope) of $PEGNB_{CA}$ (8-arm, 10 kDa) of 10 wt% $PEGNB_{CA}$ crosslinked with PEG8SH at R=0.8 with different concentrations of PEG-fluorescein-thiol (3.4 kDa). (B) Calculated fluorescent intensity from images of hydrogels.

Supporting tables

Table S1. Derived values from one-phase decay fitting of changes in normalized G' of 5 wt% PEGNB (8-arm, 20 kDa) hydrogels crosslinked with PEG4SH at R=1 when incubated at different pH buffers.

pH	$k_{hydrolysis}$ (d^{-1}) 95% CI	R^2
3	0.01755 (0.01456 to 0.02072)	0.8332
7.4	0.01636 (0.01500 to 0.01775)	0.9369
12	195.3 (144.6 to N/a)	0.9976

Table S2. Derived values from one-phase decay fitting of changes in normalized G' of 5 wt% $PEGNB_{CA}$ (8-arm, 20 kDa) hydrogels crosslinked with PEG4SH at R=1 when incubated at different pH buffers.

pH	$k_{hydrolysis}$ (d^{-1}) 95% CI	R^2
3	0.1459 (0.1304 to 0.1639)	0.9683
7.4	0.1501 (0.1422 to 0.1587)	0.9925
12	0.1732 (0.1590 to 0.1891)	0.9846

Table S3. Derived values from one-phase decay fitting of changes in normalized G' of 5 wt% $PEGNB_{CA}$ (8-arm, 20 kDa) hydrogels crosslinked with PEG4SH at R=1 when incubated at different temperatures.

Temperature (°C)	$k_{hydrolysis}$ (d^{-1}) 95% CI	R^2
25	0.01395 (0.1314 to 0.01494)	0.9419
37	0.1501 (0.1421 to 0.1587)	0.9925

Table S4. Derived values from one-phase decay fitting of changes in normalized G' of 10 wt% $PEGNB_{CA}$ (8-arm, 20 kDa) hydrogels crosslinked either enzymatically or through light-initiated polymerization with PEG8SH at R=1.

Crosslinking Method	$k_{hydrolysis}$ (d^{-1}) 95% CI	R^2
Light	0.08578 (0.08337 to 0.08829)	0.9911
Enzymatic	0.08243 (0.07701 to 0.08836)	0.9773

Table S5. Derived values from one-phase decay fitting of changes in normalized G' of $PEGNB_{CA}$ (8-arm, 20 kDa) hydrogels crosslinked with different thiol crosslinkers at different macromer content.

[Macromer] (wt%)-Thiol Crosslinker	$k_{hydrolysis}$ (d^{-1}) 95% CI	R^2
5 wt%-**DTT**	0.3874 (0.3483 to 0.4315)	0.9610
5 wt%-**PEG4SH**	0.1812 (0.1717 to 0.1913)	0.9841
5 wt%-**PEG8SH**	0.07567 (0.07228 to 0.07928)	0.9718
10 wt%-**DTT**	0.3565 (0.3366 to 0.3779)	0.9864
10 wt%-**PEG4SH**	0.1817 (0.1713 to 0.1930)	0.9830
10 wt%-**PEG8SH**	0.08578 (0.08337 to 0.08829)	0.9911
20 wt%-**DTT**	0.3005 (0.2792 to 0.3240)	0.9821
20 wt%-**PEG4SH**	0.1557 (0.1463 to 0.1659)	0.9789
20 wt%-**PEG8SH**	0.08477 (0.8136 to 0.08838)	0.9824

***References*:**

1. Y. Liu, R. Lipowsky and R. Dimova, *Langmuir*, 2012, **28**, 3831-3839.
2. C.-C. Lin and A. T. Metters, *Advanced drug delivery reviews*, 2006, **58**, 1379-1408.