Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Dually degradable click hydrogels for controlled degradation and protein release

Prathamesh M. Kharkar,[a] April M. Kloxin,*[ab] and Kristi L. Kiick*[acd]

[a]Department of Materials Science and Engineering, University of Delaware, Newark, DE 19716, USA. E-mail: akloxin@udel.edu; kiick@udel.edu

[b]Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE 19716, USA

[c]Biomedical Engineering, University of Delaware, Newark, DE 19716, USA

[d]Delaware Biotechnology Institute, University of Delaware, Newark, DE 19716, USA

Supplementary Information

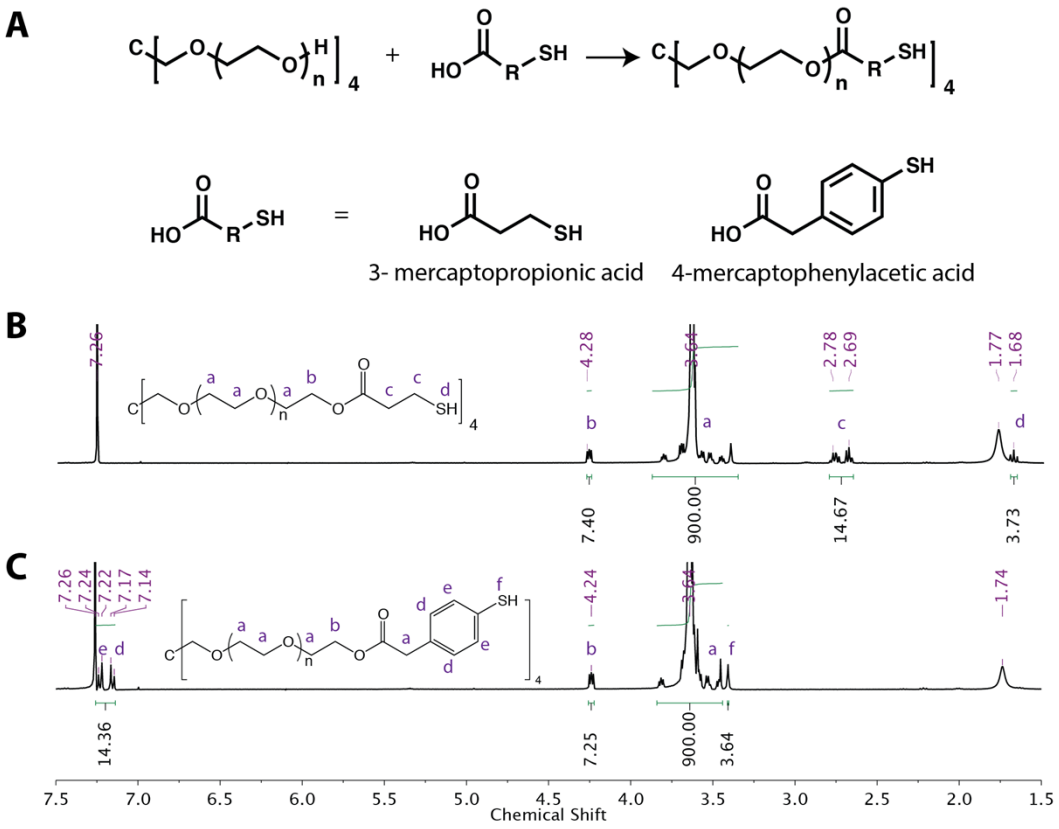
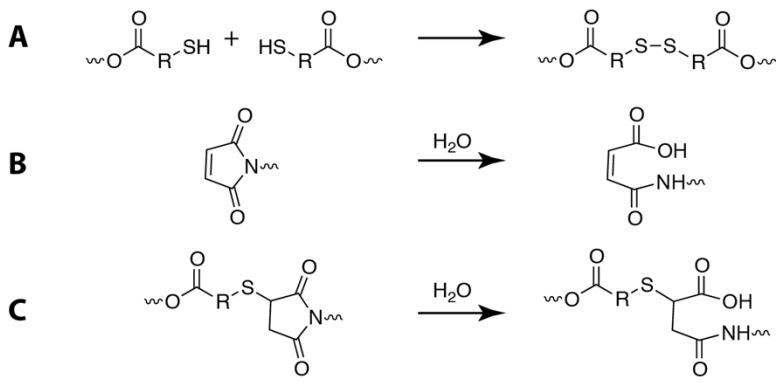


Fig. S1. Functionalization of PEG. A) Reaction schematic for mercaptoacid esterification of PEG. ^{1}H NMR spectra for 4-arm PEG functionalized with **B)** 3-mercaptopropionic acid and **C)** 4-mercaptophenylacetic acid. The functionality was calculated using the integration area of the proton (labeled b) neighboring the ester linkage (functionality: MP = 92%, MPA = 90%).



Scheme S1. Potential side reactions. Hydrogel precursor solutions can undergo **A)** disulfide formation and **B)** maleimide ring hydrolysis, which can impact the effective stoichiometry of available SH:MI groups for hydrogel formation. **C)** Thioether succinimides can undergo ring hydrolysis, making the thioether succinimide linkage unavailable for thiol exchange reactions. However, the rate of ring opening is significantly slower as compared the thiol exchange (order of magnitude different). In addition, ring hydrolysis does not result in breaking of crosslinks and subsequent hydrogel degradation.

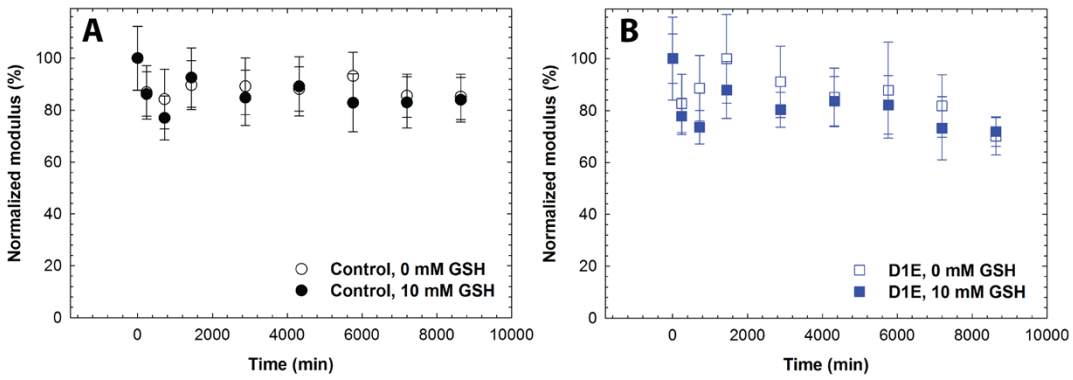


Fig. S2. Stability of Control and D1E hydrogels under non-reducing and reducing microenvironment. The effect of local microenvironment (0 and 10 mM GSH) on **A) Control** and **B) D1E** hydrogel was studied by monitoring the decrease in storage modulus at discrete time points. The initial decrease in moduli for **Control** and **D1E** in 0 mM GSH and 10 mM GSH can be attributed to equilibrium swelling. **D1E** hydrogels, compared to the **Control**, show a relatively larger decrease in moduli, confirming their slow degradation due to hydrolysis. Overall, these data indicate that there were no significant changes in moduli for reducing vs. non-reducing conditions for **Control** and **D1E** hydrogels at respective time points. The data shown illustrate the mean (n = 6), with error bars showing the standard error.

Degradation kinetics

The rate of degradation for **D2ER** hydrogels was evaluated by monitoring storage modulus (G) as a function of time. **D2ER** hydrogels degraded in 10 mM GSH microenvironment are discussed here as an example of the approach used for this analysis. According to the theory of rubber elasticity,[1] material modulus is defined by the following equation:

$$G = \frac{\rho RT}{M_c} Q^{-1/3} \qquad \ldots(S4)$$

where ρ is the density of the polymer, R is the universal gas constant, T is the temperature, M_c is the molecular weight between the crosslinks for the equilibrium swollen gel, and Q is the volumetric swelling ratio. Since we define the degradation of the hydrogel as the scission of network crosslinks (NC), the rate of hydrogel degradation can be described by the following differential equation:

$$-\frac{d[NC]}{dt} = k\,[NC][GSH][H_2O] \qquad \ldots(S5)$$

However, since the concentration of thiols from GSH is more than 2 orders of magnitude greater than that of the thiols from the **D2ER** hydrogels, the concentration of GSH in the sink can be assumed to be constant throughout the experiment time period. Similarly, the amount of water in the sink during the experiment timeframe can be assumed as constant. Consequently, the rate

expression can be simplified to describe this pseudo first order reaction as shown below.

$$-\frac{d[NC]}{dt} = k_{eff}\,[NC]$$

…(S6)

The rate law was obtained by integrating this differential equation (S6) for time from 0 to t and a concentration of network crosslinks from $[NC]_0$ to [NC], arriving at equation S7.

$$[NC] = e^{-k_{eff}t}\,[NC]_0$$

…(S7)

The network crosslinks are directly proportional to hydrogel crosslink density (ρ_x), and hence from equation S4 and S7, we obtain direct correlation between storage modulus and hydrogel degradation rate constant.

$$G \propto \rho_x \propto e^{-k_{eff}t}$$

…(S8)

Following this method, similar generalizations were made for other sink conditions and are summarized in Table 1. The rate constant for each reaction, k_{eff}, was determined by linear regression using initial parameter estimate functions (SigmaPlot v11, total number of fits = 2000, maximum number of iterations = 200, and stepsize = 1). The results of these regressions are shown in Figures S3 and S4. **Control** hydrogels under non-reducing as well as reducing conditions exhibited limited changes in G' highlighting their non-degradability. **D1E**

hydrogels exhibited degradation via ester hydrolysis as indicated by decrease in G' in both non-reducing and reducing conditions (Fig. S4).

Table S1: D2ER hydrogel degradation kinetics

Sink condition	**Limiting parameters influencing rate of degradation**	**Rate law**	**k_{eff}**
0 mM GSH	Number of crosslinks	$-\frac{d[NC]}{dt} = k_{eff}\,[NC]$	1.37 x 10^{-5} /min
0.01 mM GSH	Number of crosslinks, GSH concentration	$-\frac{d[NC]}{dt} = k_{eff}\,[NC][GSH]$	5.03 x 10^{-6} /mM min
10 mM GSH	Number of crosslinks	$-\frac{d[NC]}{dt} = k_{eff}\,[NC]$	1.75 x 10^{-3} /min

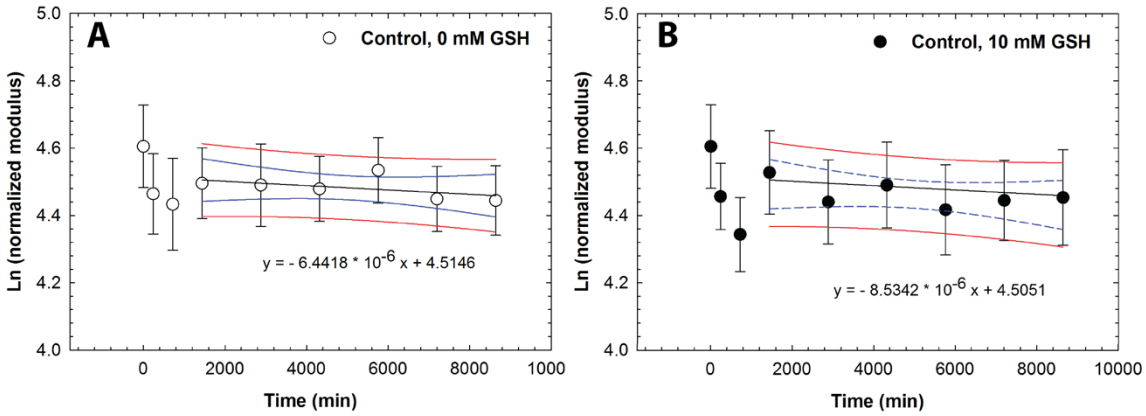


Fig. S3. Regression analysis for Control hydrogel. Changes in mechanical properties were studied by monitoring storage moduli of **Control** hydrogels suspended under A) non-reducing and B) reducing conditions. The initial decrease in normalized moduli can be attributed to equilibrium swelling. The regression analysis was carried out for timepoints after ~24 hours. The linearity of data points with limited slope (slope with standard error for non-reducing condition = $6.64 \times 10^{-6} \pm 5.24 \times 10^{-6}$ and for reducing condition = $8.53 \times 10^{-6} \pm 6.06 \times 10^{-6}$) indicates that the **Control** hydrogels were stable under both conditions (i.e., no degradation). The data shown illustrate the mean (n = 6), with error bars showing the standard error. Initial time points till 1440 minutes were excluded in regression analysis due to initial swelling causing decrease in moduli. Black line indicates linear fit. Blue and red lines indicate 95% confidence and prediction bands.

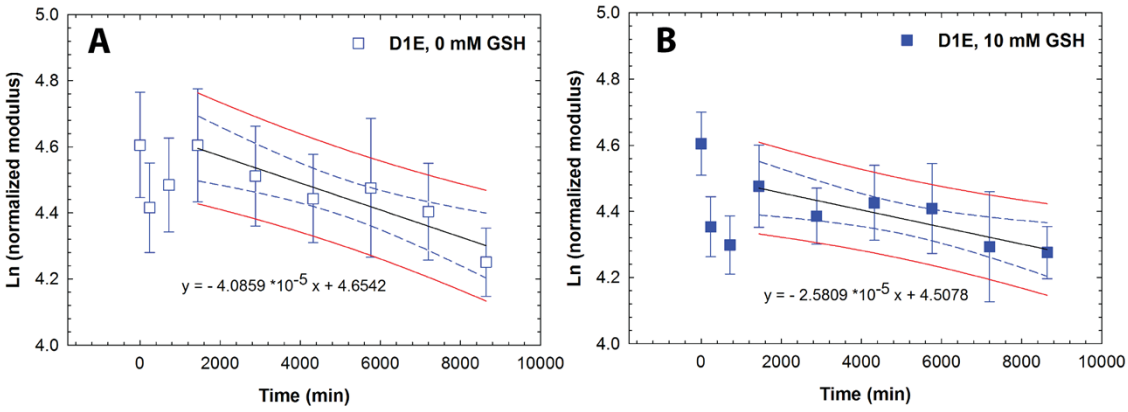


Fig. S4. Regression analysis for D1E hydrogel. Changes in mechanical properties were studied by monitoring storage moduli of **D1E** hydrogel suspended under A) non-reducing and B) reducing conditions. The initial decrease in normalized moduli can be attributed to equilibrium swelling. The regression analysis was carried out for timepoints after ~24 hours. The linearity of degradation curve with slope indicates that the **D1E** hydrogels showed degradation due to ester hydrolysis (slope with standard error for reducing condition = $4.09 \times 10^{-5} \pm 8.12 \times 10^{-6}$ and non-reducing condition = $2.58 \times 10^{-5} \pm 6.71 \times 10^{-6}$). Comparison of rate of degradation based on regression analysis and slope values for **D1E** and **Control** using Student's t-test indicated statistically significant differences highlighting role of ester linkages in degradation of **D1E** hydrogels. The data shown illustrate the mean (n = 6), with error bars showing the standard error. Initial time points till 1440 minutes were excluded in regression analysis due to initial swelling causing decrease in moduli. Black line indicates linear fit. Blue and red lines indicate 95% confidence and prediction bands.

Values for coefficient of determination for non-reducing and reducing conditions were found to be 0.86 and 0.79 respectively.

Cumulative protein release

The cumulative protein release (R) at each time point was calculated using the following equation:

$$R_t = V_r C_r + \sum_{i=1}^{n} (V_{m_i} C_i)$$

where V_m and V_r indicate amount of sink solution used for release measurement and remaining volume of sink solution respectively (i.e. total volume of sink, $V = V_r + V_m$) at each time point measurement, C is the concentration of released BSA-488 obtained using fluorometry and calibration curve, and i is the experiment time points.

Mesh size calculation

The mesh size was calculated using the Flory-Rehner equation[2] as shown below:

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{(\bar{v}/V_1)(\ln(1 - v_2) + v_2 + \chi_1 v_2^2)}{v_2^{1/3} - (v_2/2)}$$

…(S1)

where $\bar{M}_c$ is average molecular weight between crosslinks, $\bar{M}_n$ is the number average molecular weight of the uncrosslinked macromolecular chain, $\bar{v}$ is the specific volume of the polymer, $V_1$is the molar volume of the solvent (18 cm^3/mol for water), v_2 is the equilibrium volume fraction ($v_2 = Q^{-1}$), and χ_1 is the polymer-solvent interaction parameter (0.45 for PEG-water system).[3] The unperturbed root-mean-square end-to-end distance$((\bar{r}_0^2)^{1/2})$ was calculated by:

$$(\bar{r}_0^2)^{1/2} = lC_n^{1/2}\left(\frac{2\bar{M}_c}{M_r}\right)^{1/2}$$

…(S2)

where l is the average bond length (1.46 Å), C_n is the characteristic ratio for PEG, taken here as 4, and M_r is the molecular weight of the polymer repeat unit (44 g/mol for PEG). The mesh size was calculated using the following equation,[4]

$$\xi = v^{-\frac{1}{2}^{1/3}}(\bar{r}_0^2)^{1/2}$$

…(S3)

Effective diffusion coefficient calculation

Effective diffusion coefficient was computed as previously reported.[5] Briefly, the effective diffusion coefficient (D_e) for the initial time period during which equilibrium swelling occurs was estimated using a modified form of Fick's law as shown below,[6, 7] assuming uniform initial drug concentration within the hydrogel:

$$\frac{M_t}{M_\infty} = 4\left(\frac{D_e t}{\pi\delta^2}\right)^{1/2} = k'\sqrt{t}$$

where M_t and M_∞ are the absolute concentration of released cargo at time t and infinite time, respectively, δ is the thickness of hydrogel, and k' is a constant. The value of k' was obtained by plotting M_t/ M_∞ versus $\sqrt{t}$.

Reference

1. L. R. G. Treloar, *The physics of rubber elasticity*, Oxford University Press, 1975.
2. Flory and P. J., *Principles of polymer chemistry*, Cornell University Press, 1953.
3. J. Brandrup and E. H. Immergut, *Polymer Handbook*, Wiley-Interscience, New York, 1975.
4. T. Canal and N. A. Peppas, *J Biomed Mater Res*, 1989, 23, 1183-1193.
5. S. P. Zustiak and J. B. Leach, *Biotechnol Bioeng*, 2011, 108, 197-206.
6. J. Siepmann and N. A. Peppas, *Adv Drug Deliver Rev*, 2012, 64, 163-174.
7. J. Siepmann and F. Siepmann, *Int J Pharm*, 2008, 364, 328-343.