Supplementary Information (SI) for RSC Applied Polymers.
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Supporting Information

Sonication labile PEG-based hydrogel system for biological component suspension and subsequent degradation

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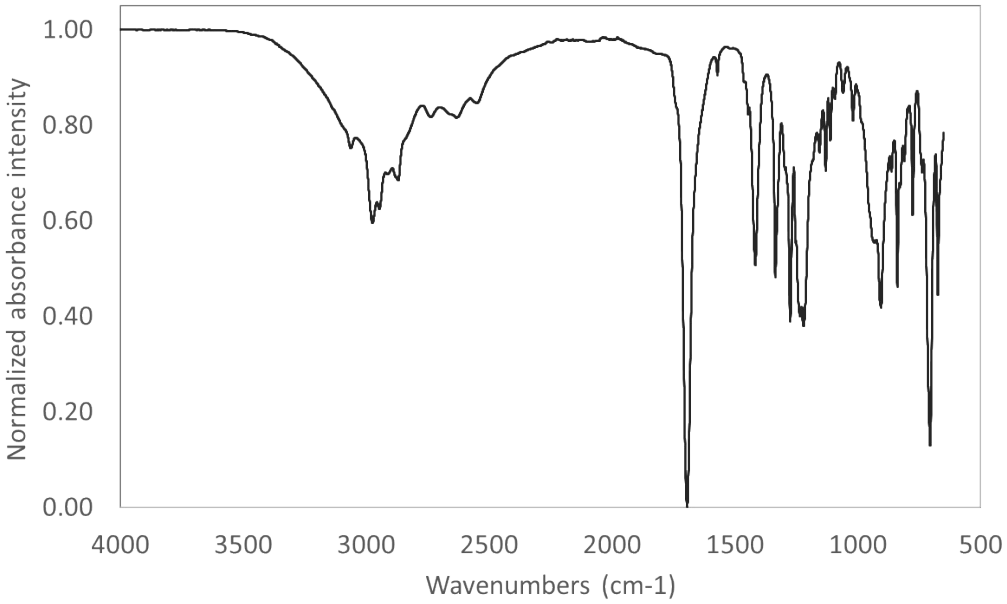
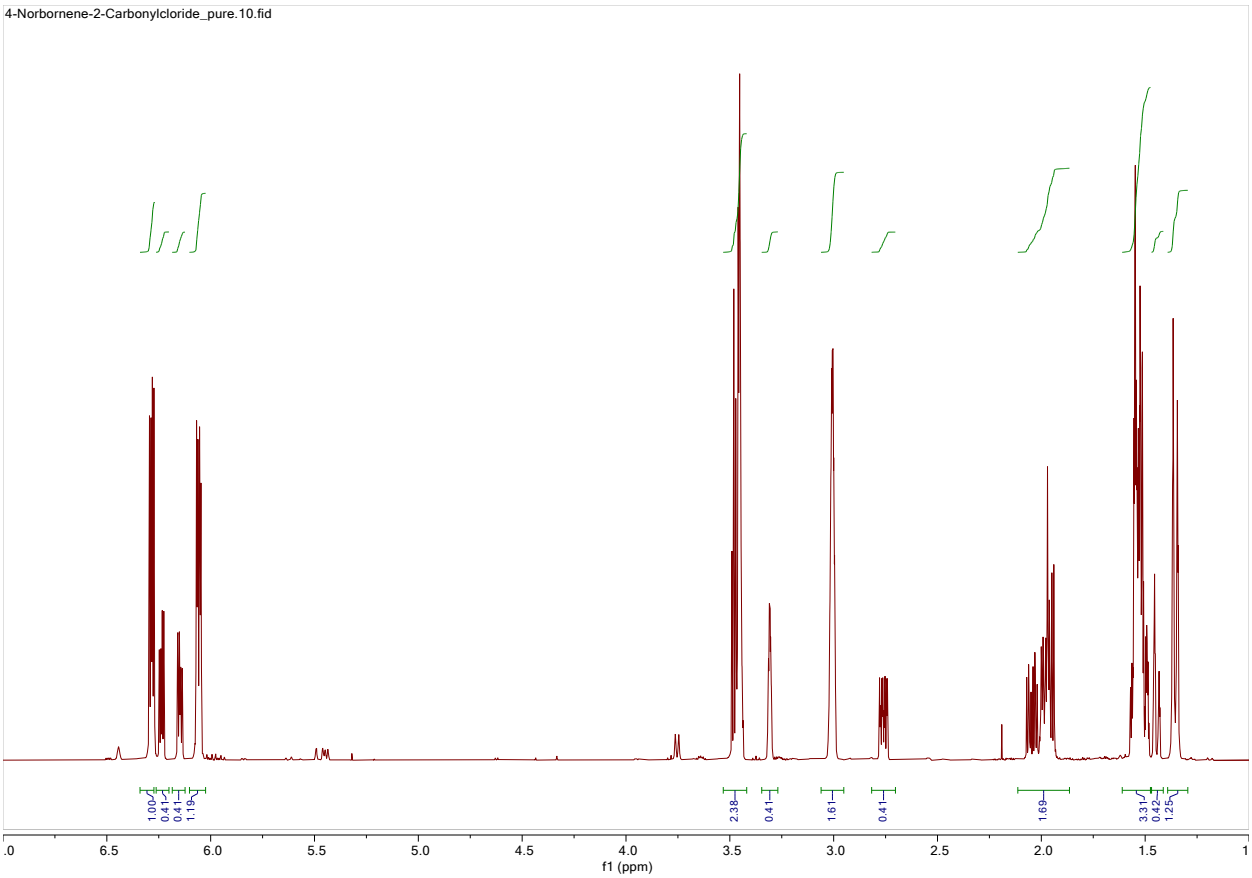
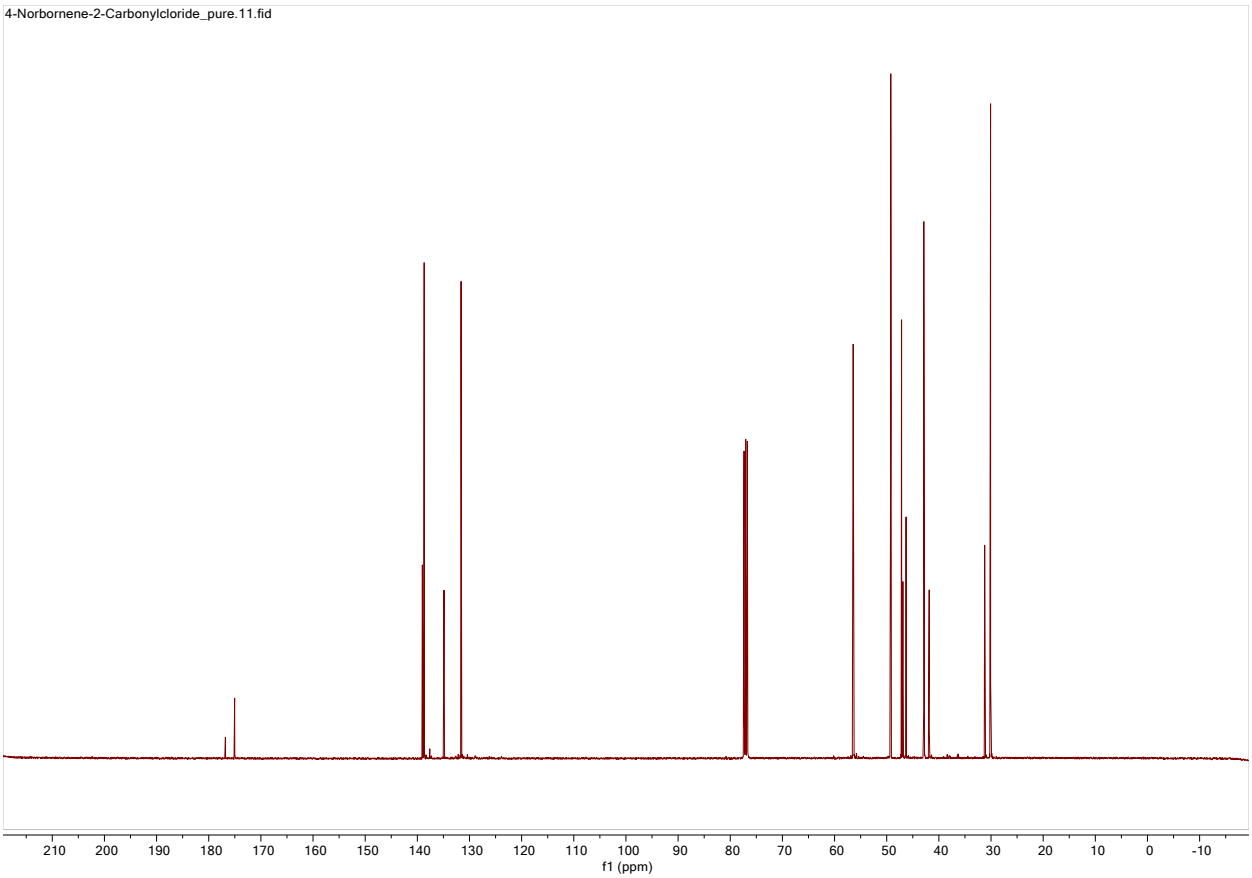


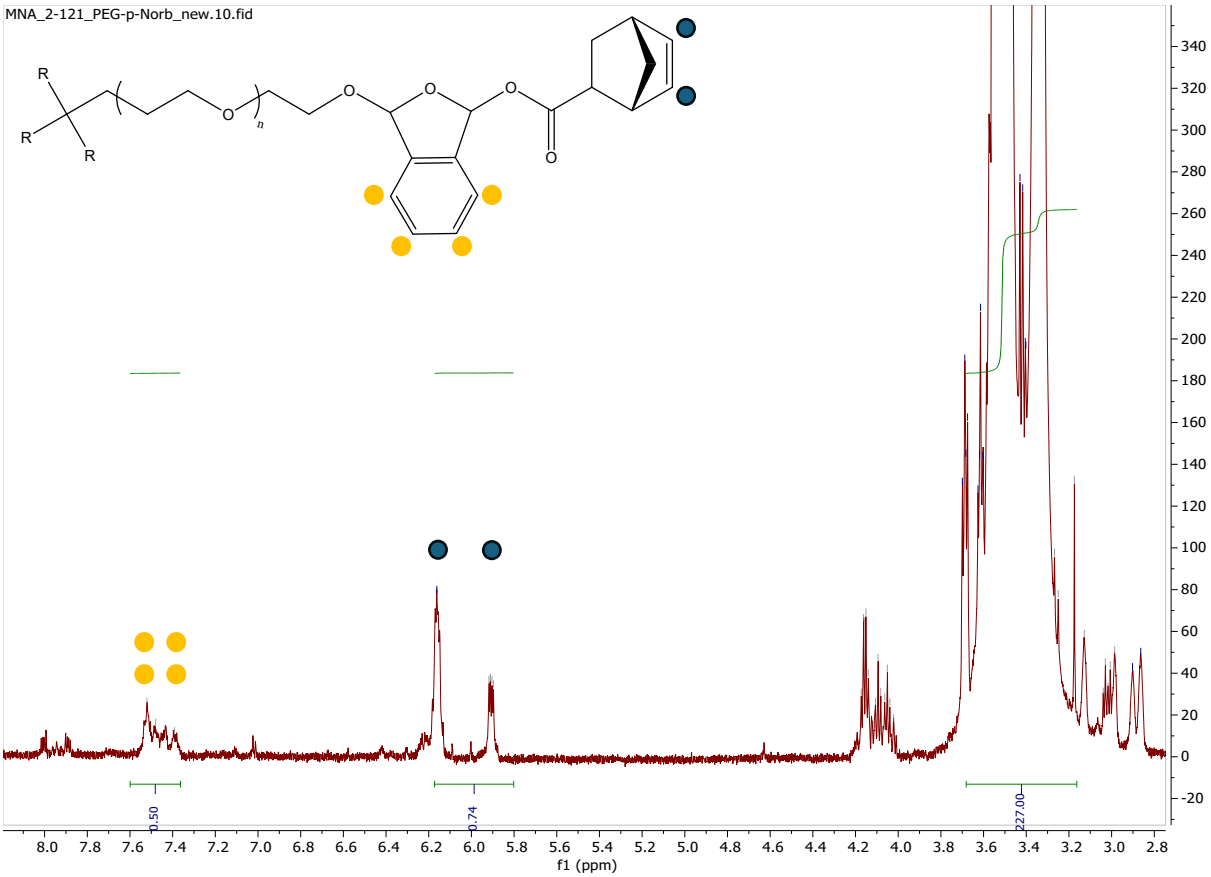
SI Figure 1. FT-IR spectra of 5-norbornrne carboxylic acid (endo, exo mixture). (cm^{-1}, u): 3139-3065 (=CH), 3000- 2832 (CH aliphatic), 1790 (C=O), 732 (C-Cl).



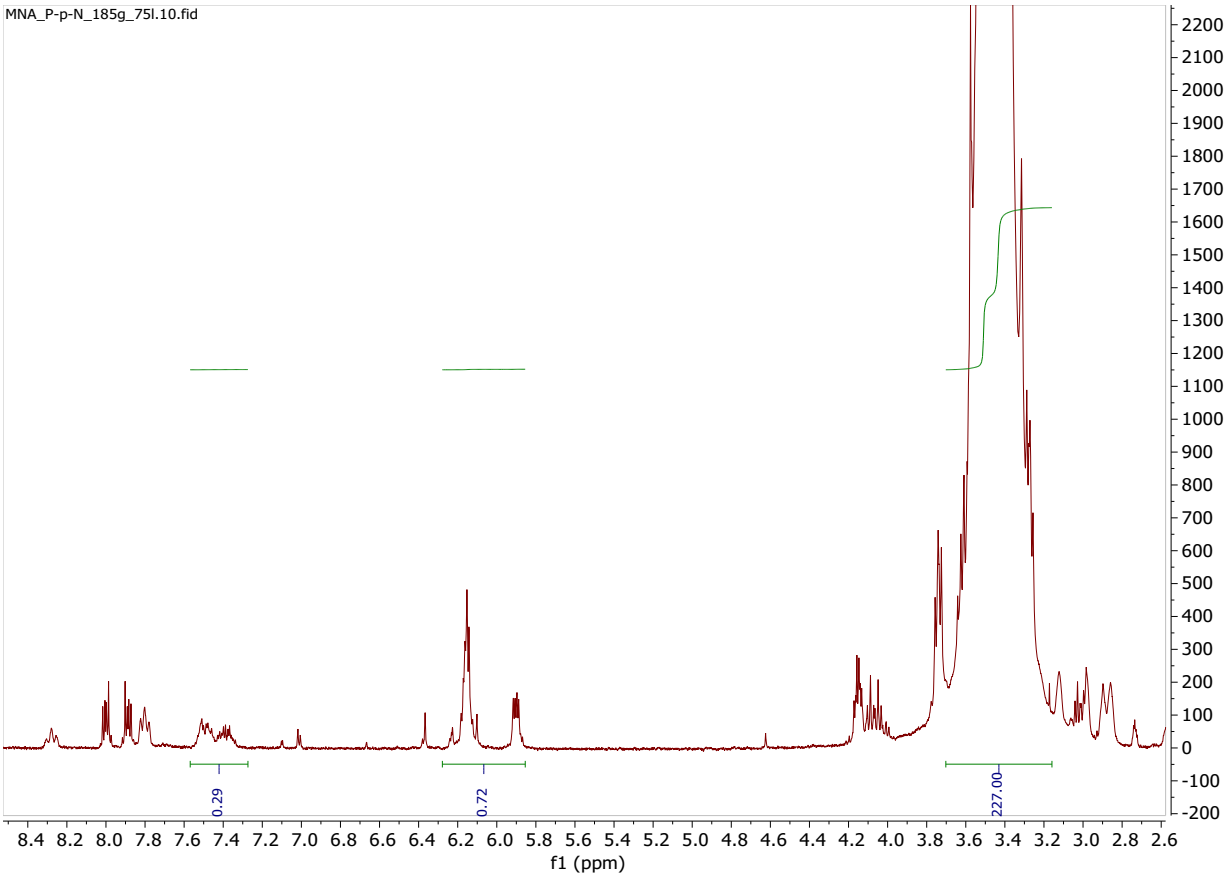
SI Figure 2. ^{1}H NMR (400 Hz, $CDCl_3$) of norbornene acyl chloride. Indicates that the product is a mixture of endo isomer (71%) and exo isomer (29%). δ: 6.27–6.29 (m, 1H, =CH, endo), 6.23–6.25 (m, 0.41H, =CH, exo), 6.16–6.14 (m, 0.41H, =CH, exo), 6.04–6.07 (m, 1H, =CH, endo), 3.44–3.49 (m, 2H, endo), 3.32-3.29 (m, 0.41H, exo), 3.00 (s, 1.6H), 2.74–2.78 (m, 0.41H, exo), 2.00–2.07 (m, 0.41H, exo), 1.93–1.99 (m, 1H, endo), 1.48–1.57 (m, 3H), 1.43–1.46 (d, 0.41H, exo), 1.34–1.37 (d, 1H, endo).



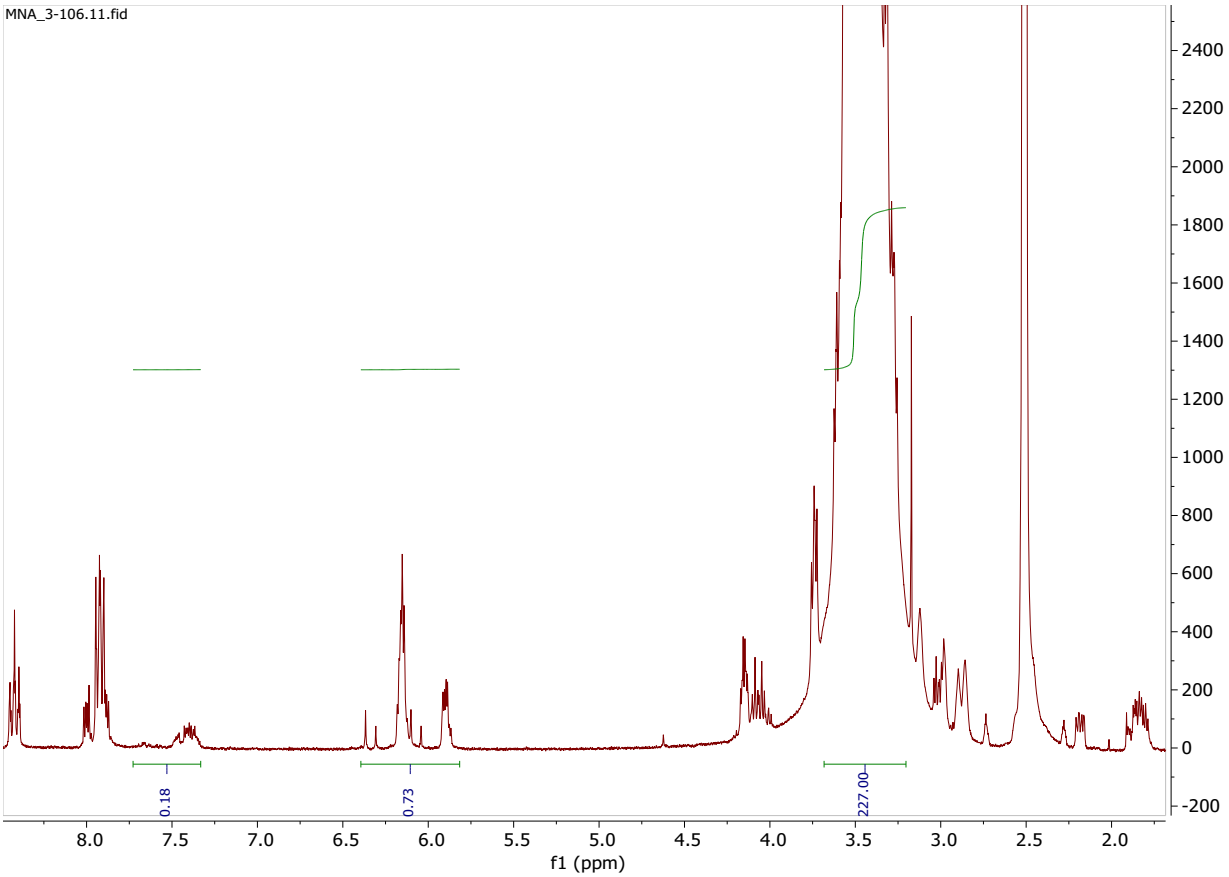
SI Figure 3. ^{13}C {1HNMR (400 Hz, $CDCl_3$) of norbornene acyl chloride. δ: 176.8 (C=O, exo), 175.0 (C=O, endo), 139.0 and 134.9 (2 C=C, exo) and 138.5 and 131.9 (C=C, endo), 56.4 (CH 2 , endo), 56.3 (CH 2 , exo), 49.2 and 47.1 (2CH, endo), 46.9 and 46.3 (2CH, exo), 42.9 (CH, endo), 41.8 (CH. exo), 31.2 (CH, exo), 30.0 (CH, endo).



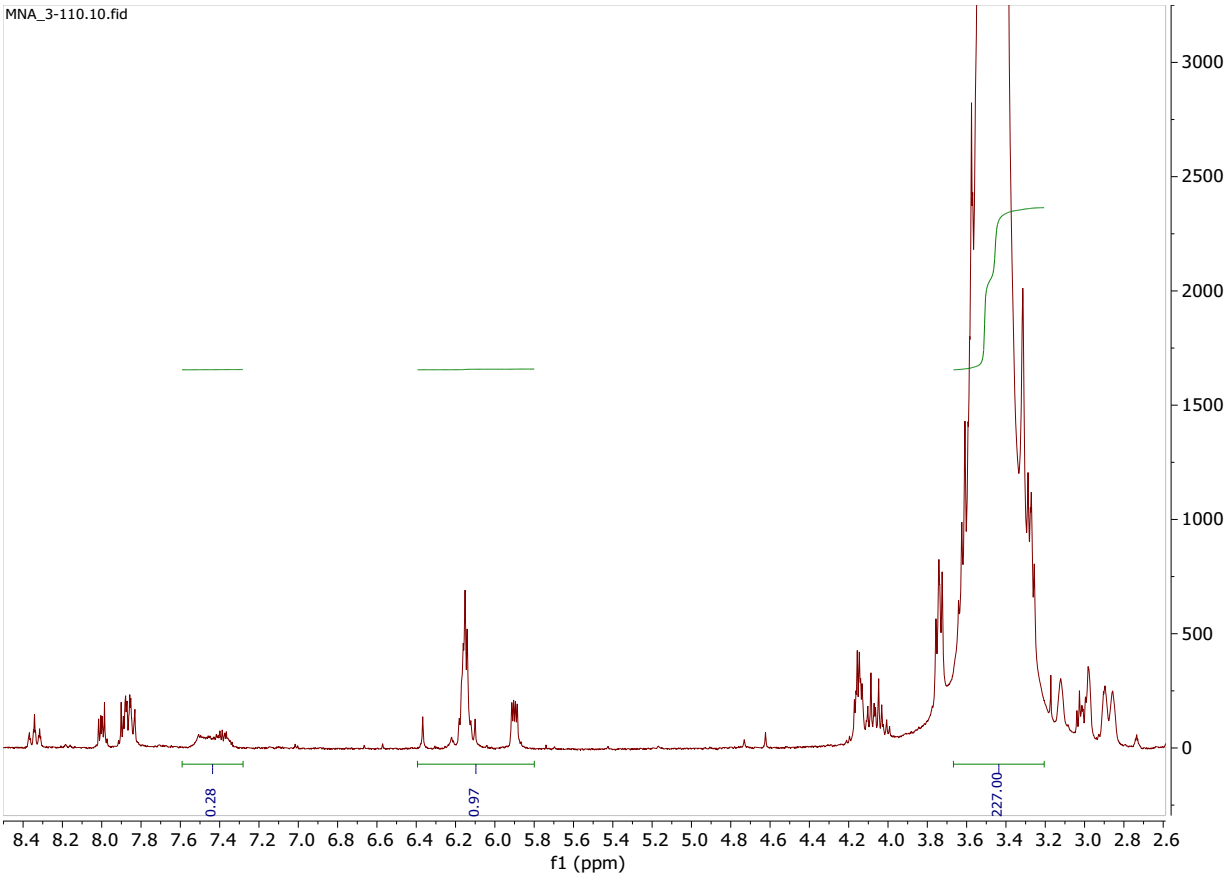
SI Figure 4. ^{1}H NMR (400 MHz, DMSO) of PEG-phthalaldehyde-norbornene used for data collection shown in this manuscript showing end group functionalization analysis. Integration suggests approximately 0.37 and 0.13 norbornene and phthalaldehyde, respectively, are added per arm of a four arm PEG. δ: 10.49, 8.65, 7.52, 7.48, 7.39, 6.16, 5.92–5.90, 4.17–4.14, 4.11–4.02, 3.70–3.68, 3.63–3.60, 3.51, 3.43–3.40, 3.34, 3.27, 3.17, 3.13, 3.04–3.01, 2.99–2.86, 2.52–2.50, 2.21–2.17, 1.90–1.80, 1.43–1.41, 1.31–1.26.



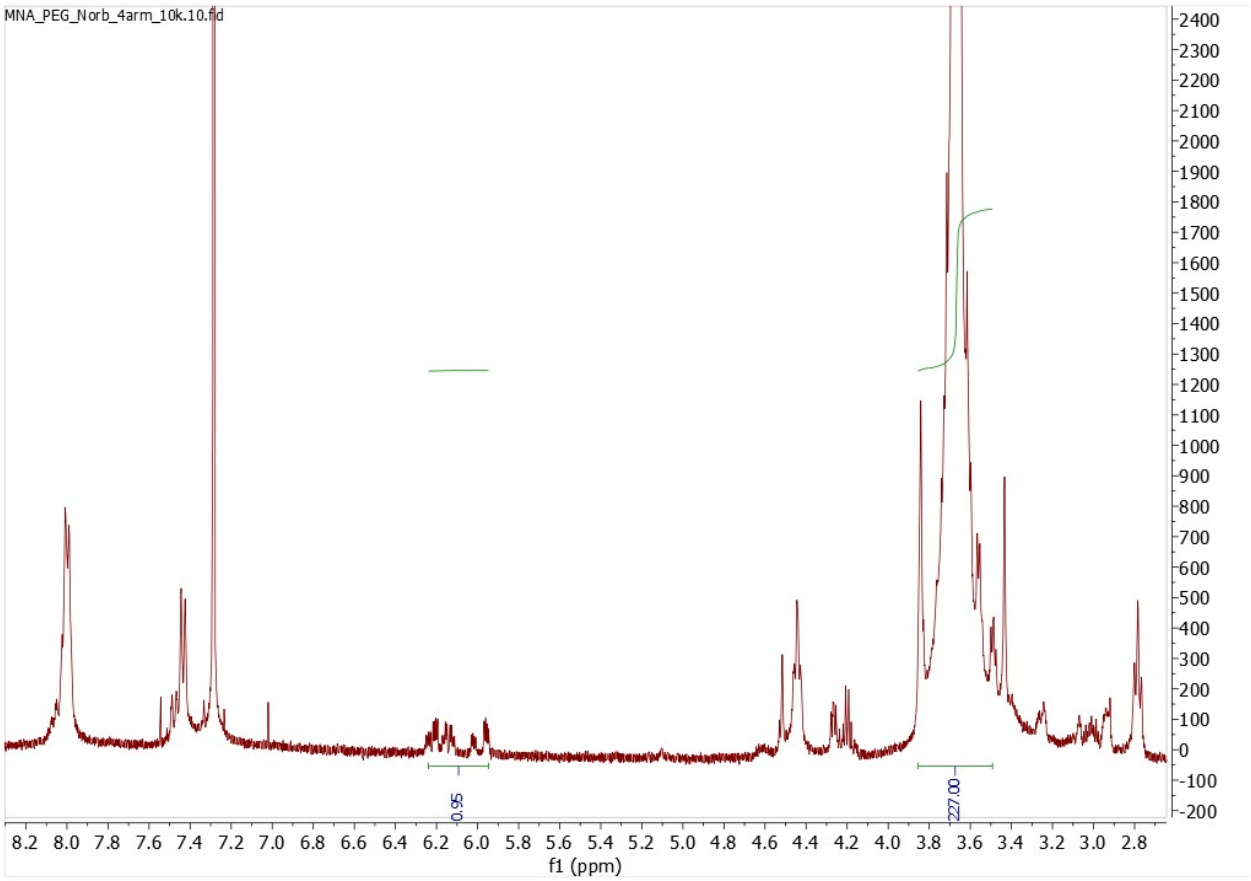
SI Figure 5. ^{1}H NMR (300 MHz, DMSO) of PEG-phthalaldehyde-norbornene showing end group functionalization analysis. Integration suggests approximately 0.36 and 0.07 norbornene and phthalaldehyde, respectively, are added per arm of a four arm PEG. δ: δ 10.48, 8.78, 8.28, 8.00, 7.80, 7.51, 7.48, 6.37, 6.16, 5.92–5.89, 4.17–4.13, 4.10–4.03, 3.76–3.72, 3.64–3.58, 3.51, 3.43–3.40, 3.34, 3.27, 3.12, 3.04–3.00, 2.98–2.90, 2.53–2.50, 2.21–2.16, 1.87–1.80, 1.43–1.40, 1.29–1.24.



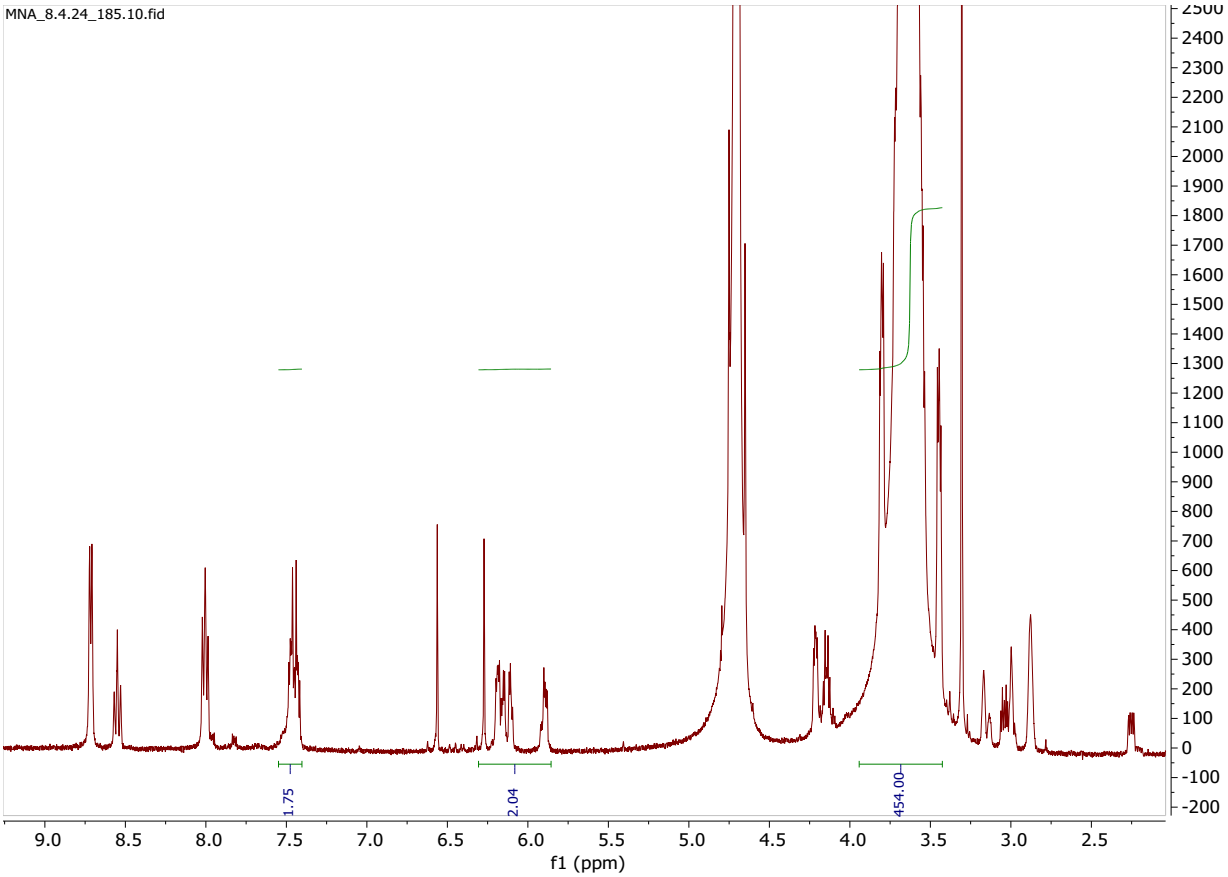
SI Figure 6. ^{1}H NMR (300 MHz, DMSO) of PEG-phthalaldehyde-norbornene showing end group functionalization analysis. Integration suggests approximately 0.37 and 0.05 norbornene and phthalaldehyde, respectively, are added per arm of a four arm PEG. δ: 10.48, 8.86–8.83, 8.45–8.39, 8.02–7.99, 7.94–7.87, 7.40, 6.37, 6.31, 6.18–6.10, 5.91–5.87, 4.17–4.14, 4.11–4.01, 3.76–3.72, 3.61, 3.51, 3.46, 3.33, 3.29, 3.17, 3.12, 3.04–3.00, 2.98, 2.86, 2.53–2.50, 2.21–2.16, 1.91–1.79, 1.40, 1.34–1.23.



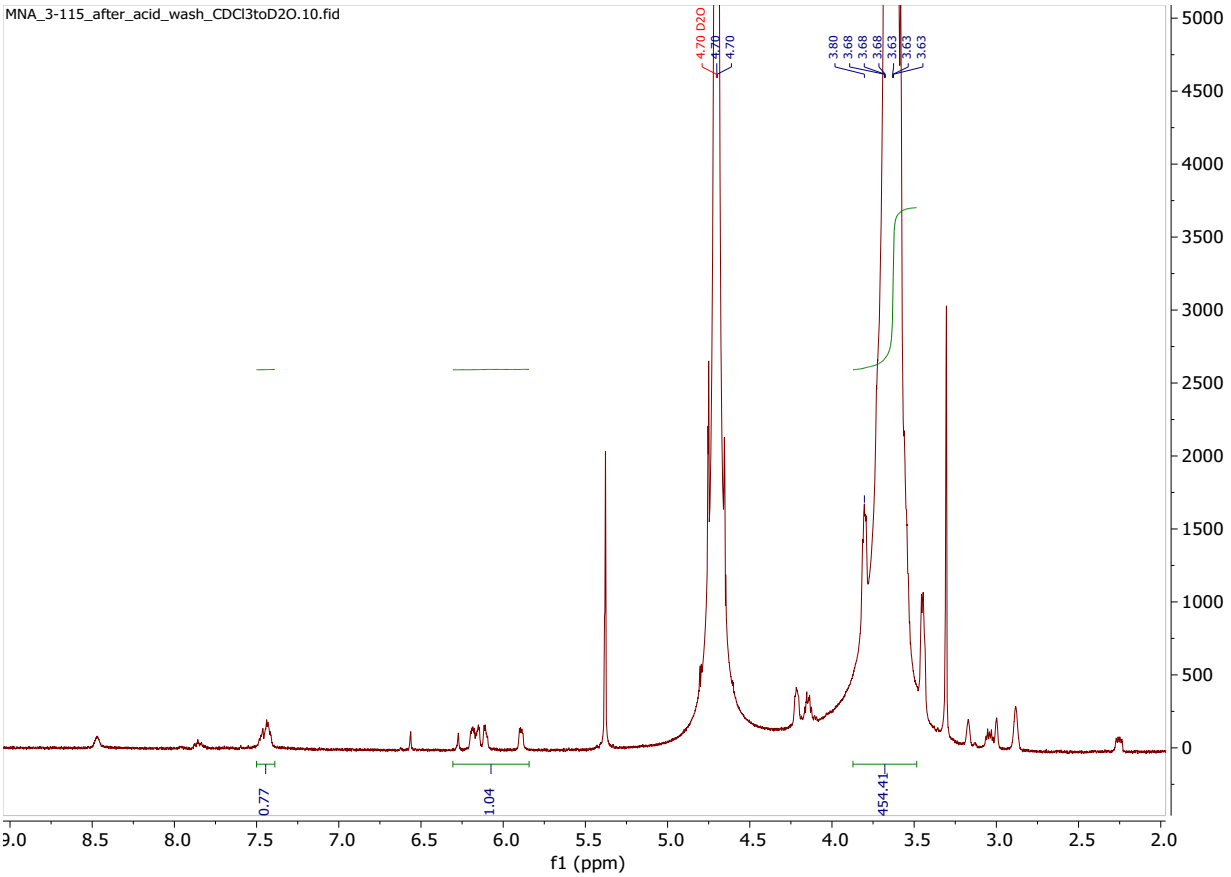
SI Figure 7. ^{1}H NMR (300 MHz, DMSO) of PEG-phthalaldehyde-norbornene showing end group functionalization analysis. Integration suggests approximately 0.49 and 0.07 norbornene and phthalaldehyde, respectively, are added per arm of a four arm PEG. δ: 10.48, 8.80, 8.34,8.02–7.97, 7.90–7.93, 7.51, 7.45, 6.37, 6.22, 6.18-6.10, 5.91–5.89, 4.62, 4.17–4.13, 4.10–4.01, 3.76–3.72, 3.64–3.61, 3.51, 3.45, 3.31, 3.29-3.26, 3.17, 3.12, 3.04–3.00, 2.98, 2.90–2.86, 2.53–2.50, 2.21–2.16, 1.90–1.79, 1.42–1.40, 1.34–1.17.



SI Figure 8. ^{1}H NMR (400 MHz, $CDCl_3$) of PEG-norbornene showing end group functionalization analysis. Integration suggests approximately 0.48 norbornenes are added per arm of a four arm PEG. δ: 8.83, 8.49, 8.01–7.99, 7.49, 7.47, 7.44, 7.42, 7.28, 4.52, 4.44, 4.27–4.25, 4.21, 4.19, 3.84, 3.72, 3.67–3.65, 3.60, 3.57–3.55, 3.50–3.47, 3.43, 3.24, 3.07, 2.80–2.77, 1.76, 1.43, 1.31, 1.28.



SI Figure 9. ^{1}H NMR (400 MHz, D_2O) of monofunctional PEG-phthalaldehyde-norbornene showing end group functionalization analysis. Integration suggests approximately 1.02 and 0.43 norbornene and phthalaldehyde, respectively per arm. δ: 8.72, 8.57, 8.55, 8.00, 7.46, 7.43, 6.56, 6.27, 6.20, 6.15, 6.09, 5.89, 4.75, 4.70, 4.65, 4.20, 4.15, 3.80, 3.72, 3.68, 3.63, 3.58, 3.54,3.45, 3.43, 3.30, 3.17, 3.00, 2.88, 1.88, 1.76, 1.30, 1.25, 1.20, 0.79.



SI Figure 10. ^{1}H NMR (400 MHz, D_2O) of monofunctional PEG-phthalaldehyde-norbornene that has been hydrolytically degraded with 1M HCl at a pH of 3. The resultant products were extracted into chloroform, the chloroform was evaporated off, and the result was solubilized in D2O for NMR. Integration of the functionalization indicates approximately 0.52 and 0.19 norbornene and phthalaldehyde, respectively per arm. δ: 8.72, 8.57, 8.55, 8.00, 7.46, 7.43, 6.56, 6.27, 6.20, 6.15, 6.09, 5.89, 4.75, 4.70, 4.65, 4.20, 4.15, 3.80, 3.72, 3.68, 3.63, 3.58, 3.54,3.45, 3.43, 3.30, 3.17, 3.00, 2.88, 1.88, 1.76, 1.30, 1.25, 1.20, 0.79.

When compared to the initial PEG-phthalaldehyde norbornene integrations (SI Figure 10), after hydrolytic degradation of the phthalaldehyde linkages the norbornene functionalization decreased by 49% and the phthalaldehyde functionalization decreased by 56%. This suggests that the majority of phthalaldehyde units attached to a PEG arm are capped by a norbornene functional group, facilitating the decrease in integration value observed in this study.

Flory-Stockmayer gel point calculations (performed for materials used in the main body of this manuscript corresponding to the NMR shown in SI Figure 4):

$F_1 = 4\ thiol$

$F_2 = 1.48\ alkene$

$r = 0.37$

Gelation

Assuming statistical distribution of functionalization on PEG arms:

f ene	Probability
0	0.158
1	0.370
2	0.326
3	0.128
4	0.019

$$F_{2,\ weightd} = \frac{\sum P_{f\ ene} *\ fene^2}{\sum P_{f\ ene} *\ f\ ene} = 2.11$$

$$Gel\ point = \frac{1}{\sqrt{r\ (F_1 - 1)(F_{2,\ weighted} - 1)}} * 100$$

Successful gelation with 90.1% conversion of alkene. Therefore, successful gelation with functionalization of NMR integration value of norbornene functionalization.

Degradation

$$Probability\ of cleavage = \frac{functionalization\ of\ phthalaldehyde}{functionalization\ of\ norbornene} = \frac{0.13}{0.37} = 0.28$$

Assumes that phthalaldehyde is capped by a norbornene (SI Figure 11), therefore any phthalaldehyde that breaks causes a reduction in the number of PEG arm's attached to the network.

$r' = r(1 - P_{cleavage}) = 0.264$

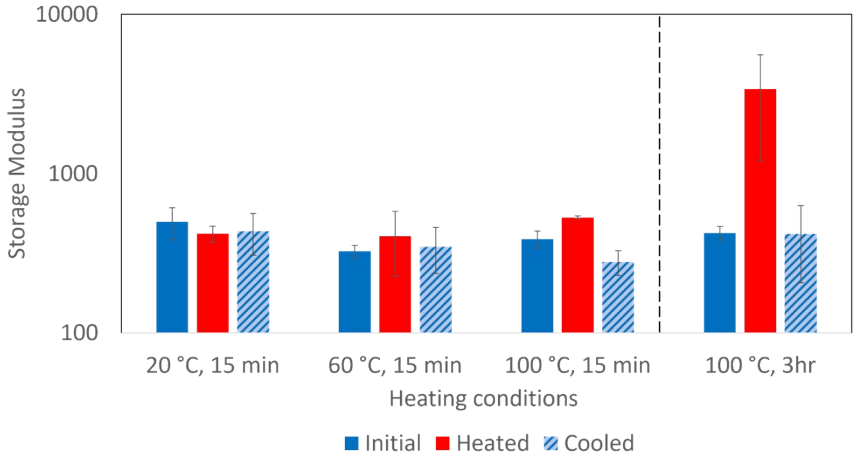
Assuming statistical distribution of functionalization on PEG arms:

f ene	Probability
0	0.292
1	0.421
2	0.228
3	0.055
4	0.005

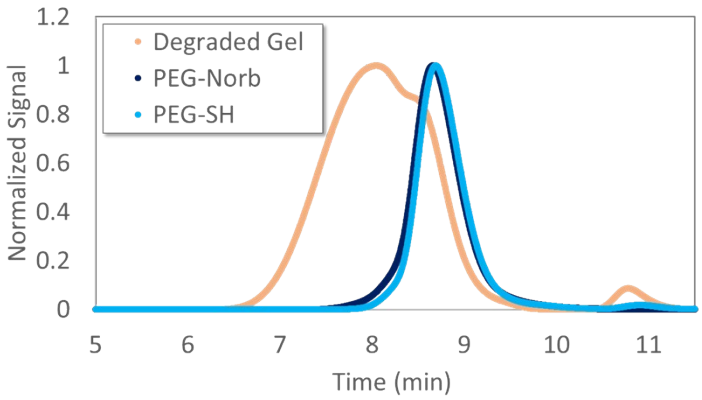
$$F_{2,\ weightd} = \frac{\sum P_{f\ ene} * \ fene^2}{\sum P_{f\ ene} * \ f\ ene} = \ 1.79$$

$$Gel\ point = \frac{1}{\sqrt{r\ (F_1 - 1)(F_{2,\ weighted} - 1)}} * 100 = 107\%$$

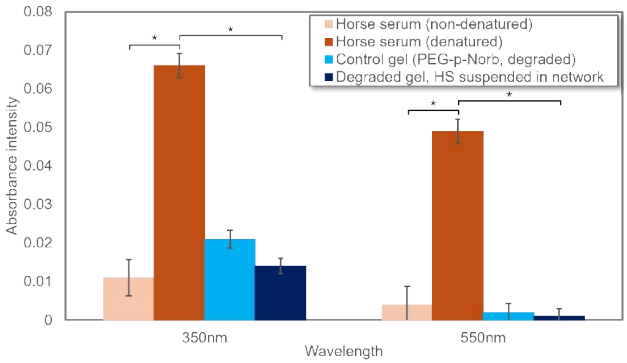
As the critical gel point is above the 100% conversion for the sonication-unaffected thioether bonds, assuming complete conversion of all norbornenes and complete degradation of all phthalaldehyde-bound thioether linkages, total degelation of the network would be expected.It is important to note the assumption of ideality for these calculations. Any nonideality in conversion would favor degelation and nonideality in degradation would disfavor degelation. The extent of nonidealities in the polymer formations is unknown, but the calculations are consistent with the results observed and reported.



SI Figure 11. Storage modulus data for PEG-p-Norbornene hydrogels (10 wt% macromer) at various heating conditions indicating the increase in storage modulus directly after heating and the return to the original storage modulus after cooling (3 hours at room temp in DI water). All samples were heated to the noted temperature for the given time while in water.



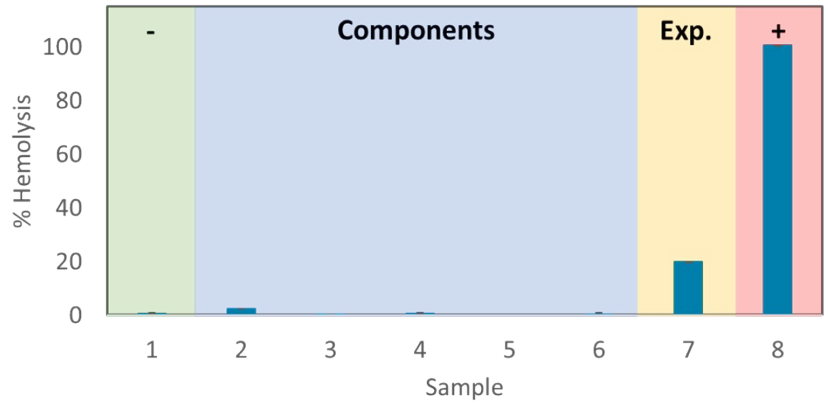
SI Figure 12. PEG-p-Norb GPC trace illustrating macromers (blue traces) and degraded products (orange trace) post 5 minutes sonication at a power output of 7W with a frequency of 20 Hz. Data from GPC-MALS experiments allow for a calculation of a number average molecular weight of the degraded gel fragments of 3.5 x 10^5 g/mol and 7.4 x 10^4 g/mol (left and right, respectively, for the orange line) while the blue peak of the PEG-Norbornene correlates to a calculated number average molecular weight of 7.3 x10^4 g/mol. Additionally, size exclusion GPC data from linear standards allows for a calculation of M_n for the degraded gel fragments equal to 20800 g/mol and 5800 g/mol (left and right respectively, for the orange line) while the blue peak has a calculated value of 6500 g/mol. The M_w calculated from the size exclusion GPC data results in values for the degraded products of 27100 g/mol and 6800 g/mol (left and right, respectively, for the orange curve) and 7700 g/mol for the PEG macromer (blue curve). Thus, demonstrating that the degradation stimulus facilitates a return towards the macromer species as well as daughter fragments that trend towards a small number of macromers being connected. The use of linear standards will introduce an error in these calculations compared to the star configuration of the PEG macromer used for these degradation experiments.



SI Figure 13. Absorbance intensity of horse serum solution (non-denatured and denatured), degraded 5 wt% hydrogel, and a degraded 5 wt% hydrogel made with horse serum solution done on plate reader. The horse serum was diluted in a 1:10 ratio with PBS (1x). Degradation conditions were 2 minutes of sonication at a power output of 7 W with a frequency of 20 Hz. The positive control of denatured horse serum was placed in an oven at 70°C for 15 minutes. At both wavelengths, the degraded hydrogel with suspended horse serum had a lower absorbance than the denatured protein solution.

Sample	PBS	RBCs (40% v/v)	PEG-p-Norb (6 wt%)	PEG-SH (6 wt%)	LAP (0.1 wt%)	UV Light (365nm, 30s)	Sonication (5W, 30 sec, 20Hz)
1	X	X					
2	X	X	X				
3	X	X		X			
4	X	X			X		
5	X	X				X	
6	X	X			X	X	
7	X	X	X	X	X	X	X
8	X	X					X

SI Table 1. Red blood cell inclusion assay sample conditions. Standards included individual macromers, photoinitiator, UV irradiation, and initiator and UV irradiation (6 wt% LAP, 365 nm, 5 mW/cm^2, 30s irradiation, 100 µm thick samples). Positive controls for hemolysis included a sample of RBCs exposed to sonication and oxidized blood. Sonication had a power output of 5 W and a frequency of 20 Hz for 30 seconds. O_2 indicates blood exposed to oxygen for an extended period of time resulting in a coagulated sample. The negative control for hemolysis was fresh RBCs. Weight percentages indicate the macromer or LAP in the red blood cell solution. Abbreviations: phosphate buffered saline (PBS), red blood cells (RBCs), polyethylene glycol phthalaldehyde norbornene (PEG-p-Norb), polyethylene glycol thiol (PEG-SH), weight percent (wt%), volume/volume (v/v), lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), ultraviolet (UV).



SI Figure 14. Percent hemolysis of red blood cells using plate reader assay (measured at 570 nm) in the PEG-p-Norb hydrogel system. The conditions for these tests are written out in SI Table 1, the negative control is indicated by the green shade, control standards by the blue shade, and positive controls by the red shade. The normal and extended sonication of hydrogels with suspended RBCs resulted in significantly less hemolysis of the RBCs compared to the positive controls. Error bars represent mean ± SD.