Supplemental Information

Rate-based approach for controlling the mechanical properties of 'thiol-ene' hydrogels formed with visible light

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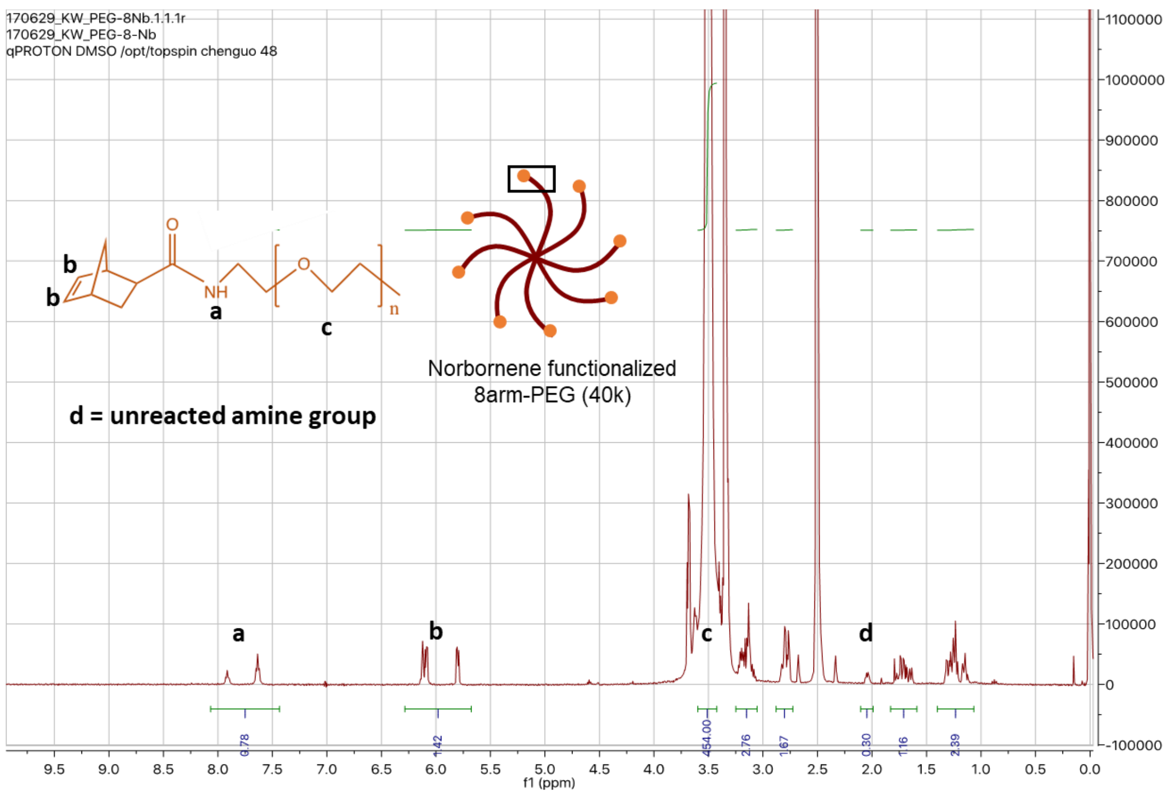


Figure S1. ^{1}H NMR of PEG-8-Nb in DMSO-d_6: 400 mHz δ 8.00 to 7.68 (m, 1H), δ 6.20 to 5.86 (m, 2H), δ 3.65 to 3.40 (m, 454H). Peak integration was normalized to the multiarm PEG backbone (setting integration of **c** to 454). Functionality was calculated by taking an average based on **a**, **b**, and **d** peak integrations (average functionality ~ 78%).

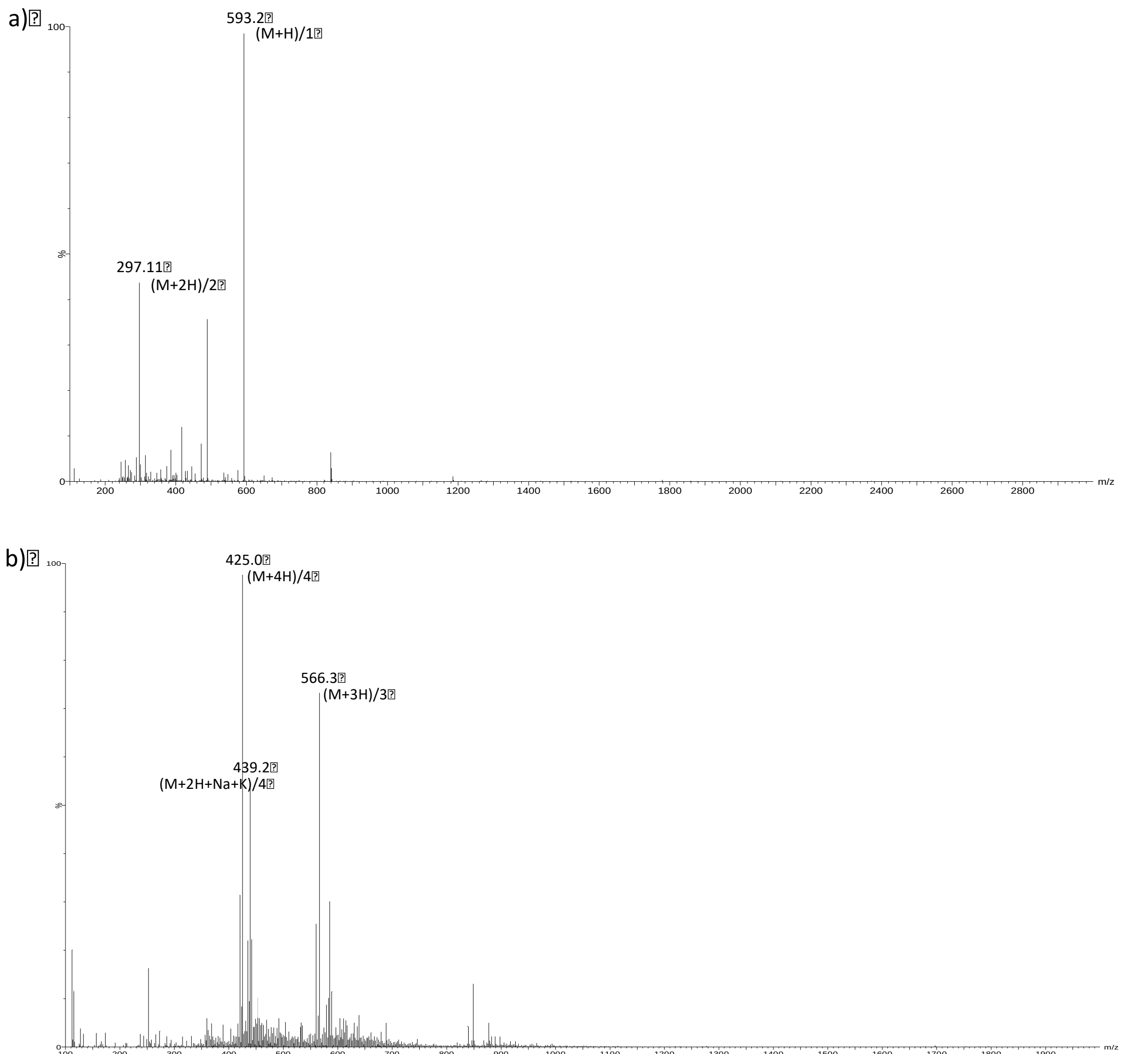


Figure S2. Mass spectrometry of peptides a) CGRGDS (MW = 592.6) and b) GCRDVPMSMRGGDRCG (MW = 1696.0).

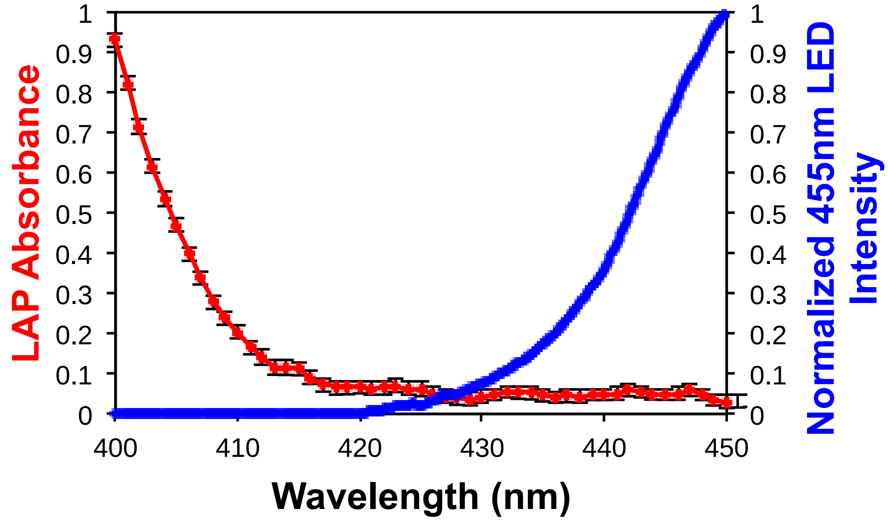
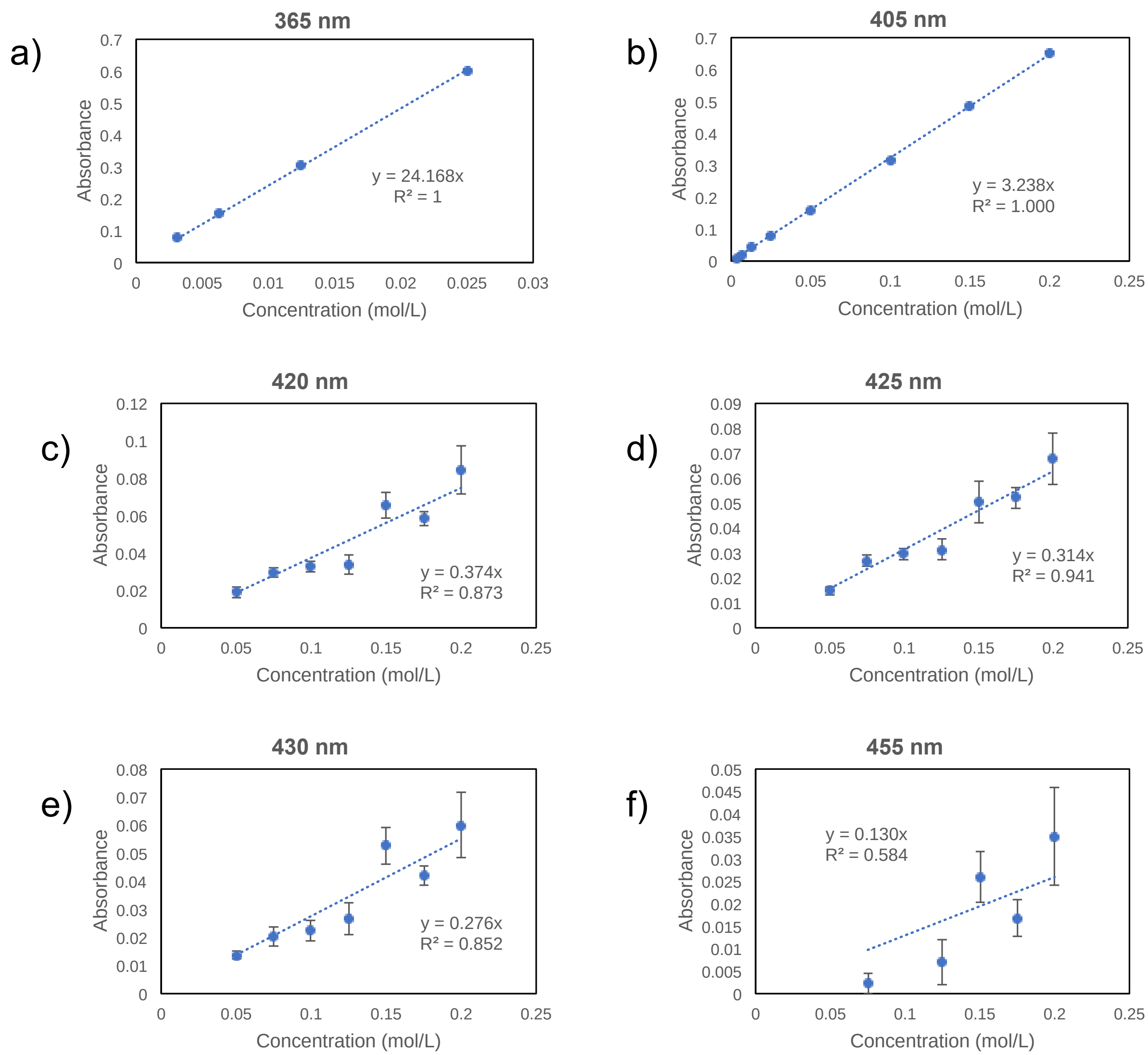


Figure S3. Comparison of LAP absorbance (200 mM in PBS) and visible light LED lamp light intensity (normalized to maximum intensity).



f)

Wavelength	365 nm	405 nm	420 nm	425 nm	430 nm	455 nm
slope	24.168	3.238	0.374	0.314	0.276	0.13
ε (L/(mol cm))	240	32	4	3	3	1

Figure S4. Light absorbance of LAP in PBS. a-f) Graphs of LAP absorbance at a particular wavelengths (365, 405, 420, 425, 430, or 455 nm) vs. concentration (LAP in PBS). g) Estimated molar absorptivity of LAP in PBS at various wavelengths, calculated using Beer's Law, where the path length = 1 mm.

Table S1. Estimated transmittance of various light wavelengths through different sample geometries: a) syringe mold (height = 1 mm) and b) MAS-NMR rotor inserts (height = 6mm), where 365 nm irradiated samples contain 2 mM LAP and visible light irradiated samples contain 4 mM LAP. Transmittance estimated using Beer's Law and measured molar absorptivity of LAP at respective wavelengths (Figure S4).

a)

Wavelength (nm)	**Concentration LAP (mM)**	**Transmittance (%)**
365	2	89.5
420	4	99.7
425	4	99.7
430	4	99.7
455	4	99.9

b)

Wavelength (nm)	**Concentration LAP (mM)**	**Transmittance (%)**
365	2	51.3
420	4	98.0
425	4	98.3
430	4	98.5
455	4	99.3

Table S2. p-values for statistical analysis of moduli for various 455nm LED bulk hydrogel formation conditions: comparisons for a) hydrogels formed at 70 $mWcm^{-2}$ for 5 minutes, b) hydrogels formed at 90 $mWcm^{-2}$ for 5 minutes, and c) hydrogels formed at 70 $mWcm^{-2}$ (red) and 90 $mWcm^{-2}$ (blue) at each wt% PEG-8-Nb, where * indicates $p < 0.05$, ** indicates $p < 0.01$, and n.s. indicates not significant. Significance was determined by two-sided Student's t-test.

a)

wt% PEG 1	wt% PEG 2	p-value	significance
6	8	0.008	**
8	10	0.361	n.s.
10	12	0.090	n.s.
12	14	0.599	n.s.
6	10	0.005	**
8	12	0.018	*
10	14	0.050	*
6	12	0.001	**
8	14	0.011	**
6	14	0.001	**

b)

wt% PEG 1	wt% PEG 2	p-value	significance
6	8	0.009	**
8	10	0.193	n.s.
10	12	0.095	n.s.
12	14	0.019	*
6	10	0.000	**
8	12	0.033	*
10	14	0.000	**
6	12	0.006	**
8	14	0.000	**
6	14	0.000	**

c)

wt% PEG 1	wt% PEG 2	p-value	significance
6	6	0.000	**
8	8	0.002	**
10	10	0.001	**
12	12	0.007	**
14	14	0.000	**

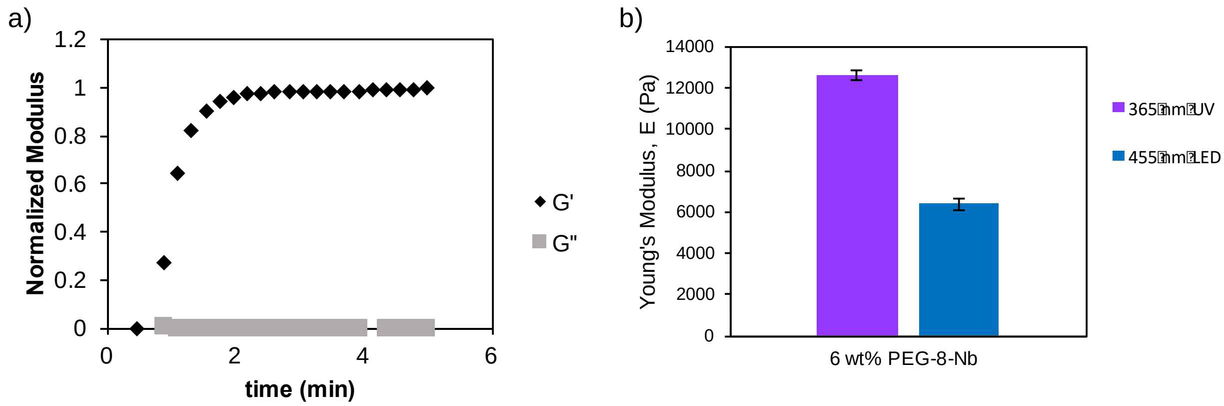


Figure S5. a) Example data of monitoring hydrogel polymerization with in situ rheometry; here, irradiation commenced at 1 minute (10 $mWcm^{-2}$ at 365 nm). b) Young's moduli of resulting hydrogels polymerized with visible light (70 $mWcm^{-2}$ at 455 nm for 5 minutes) and 365 nm (10 $mWcm^{-2}$ for 2 minutes) measured by DMA.

Figure S6. Evaluation of stiffening solution incubation time. a) Mesh size of hydrogels formed with 365 nm or visible light (455 nm centered LED) were estimated using Rubber Elasticity Theory following a previously established protocol,[1] where $\mathbf{M_s}$ is the mass of the hydrogel in the equilibrium swollen state, $\mathbf{M_d}$ is the mass of the hydrogel in the dry state, $\mathbf{Q_s}$ is the volumetric swelling ratio in the equilibrium swollen state, $\mathbf{G'_s}$ is the storage modulus in the equilibrium swollen state, and $\boldsymbol{\xi}$ is the mesh size. b) Diffusivity of 8-arm PEG 40kDa in water was estimated using the Stokes-Einstein equation, using previously a previously reported value of the hydrodynamic radius of 8-arm PEG 40kDa.[2,3] Diffusivity in hydrogels was estimated assuming hindered diffusion: briefly, the ratio of hydrogel mesh size and the hydrodynamic radius of 8-arm PEG (**r** = 5.66 nm) was calculated, and the diffusivity in water adjusted for hindered diffusion in the hydrogel based on correlations observed between these in previous reports.[1] c) Approximate time scale for diffusion was estimated as $\mathbf{L^2/D}$, assuming 1D (axial) Fickian diffusion, where **L** is half the thickness of the hydrogel (0.5 mm) and **D** is the estimated hindered diffusivity.[2]

a)

	M_s (mg)	M_d (mg)	Q_s	G'_s (Pa)	ξ (nm)
455 LED	35.2	1.2	30.1	2130	27.9
365 Omnicure	41.3	1.8	23.9	4130	25.5

b)

$$D = \frac{k_B T}{6\pi \eta r}$$

k_b	Boltzmann constant	1.38E-23 J/K
T	Temperature	298 K
η	dynamic viscosity	0.00091 Ns/m^2
r	Hydrodynamic radius	5.66E-09 m
D_w	Diffusivity in water	4.23E-11 m^2/s
D_H **(365)**	Diffusivity in hydrogel	1.1E-11 m^2/s
D_H **(455)**	Diffusivity in hydrogel	1.4E-11 m^2/s

c)

$$\tau = \frac{L^2}{D}$$

τ (365)	6.4 h
τ (455)	5.1 h

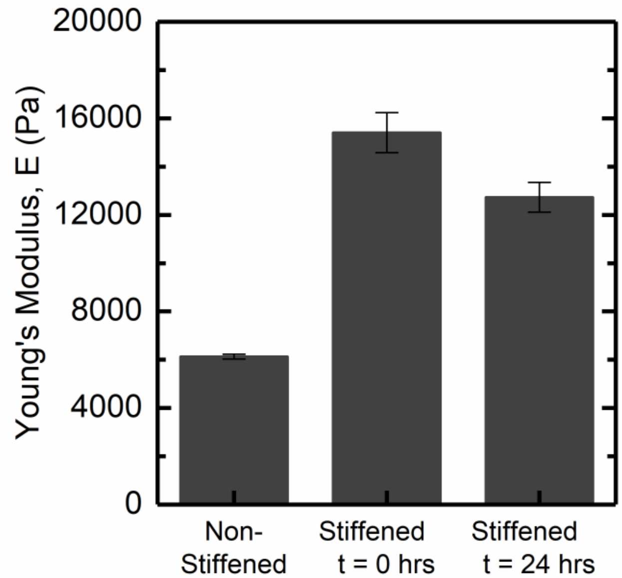


Figure S7. Moduli of hydrogels that were (i) polymerized with 455 nm light (70 $mWcm^{-2}$ for 5 minutes) (Non-stiffened) and subsequently stiffened (ii) with measurement immediately after stiffening (Stiffened t = 0 hrs) and (iii) after equilibrium swelling in PBS (Stiffened t = 24 hrs). Stiffened hydrogels at t = 0h and t = 24h are not statistically different, demonstrating that stiffened hydrogels maintain modulus after 24h of swelling in PBS.

References

1 M. S. Rehmann, K. M. Skeens, P. M. Kharkar, E. M. Ford, E. Maverakis, K. H. Lee and A. M. Kloxin, *Biomacromolecules*, 2017, **18**, 3131–3142.

2 W. M. Deen, *Analysis of transport phenomena*, Oxford University Press, New York, 2nd edition., 2012.

3 Z. Gu, Molecular-size effects of poly(ethylene glycol) doxorubicin nanocarriers on the intraductal treatment of ductal carcinoma in situ (DCIS), Rutgers, 2015.