Supporting Information

Photocleavable hydrogels for light-triggered siRNA release

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S1. Experimental Section

S1.1. Materials

Acetovanillone, ethyl 4-bromobutyrate, sodium borohydride and n-hexane were obtained from Across Organics, Thermo Fisher Scientific (New Jersey). Acryloyl chloride (AC) and phosphorus pentachloride were obtained from Alfa Aesar (Ward Hill, MA). Triethylamine (TEA), *N,N*-dimethylformamide (DMF), dichloromethane (DCM), tetrahydrofuran (THF), trifluoroacetic acid (TFA), potassium carbonate anhydrous, diethyl ether and acetone were obtained from Fisher Scientific (New Jersey). Ethanol was obtained from Pharmco-AAPER (Brookfield, CT). Nitric acid was obtained from Merck (Darmstadt, Germany). Anhydrous toluene and poly(ethylene glycol) (PEG) (Mn=4,000 Da) were obtained from Sigma Aldrich (St Louis, MO). Dialysis membrane (MWCO 3,500) was obtained from Spectrum Laboratories Inc. (Rancho Dominguez, CA).

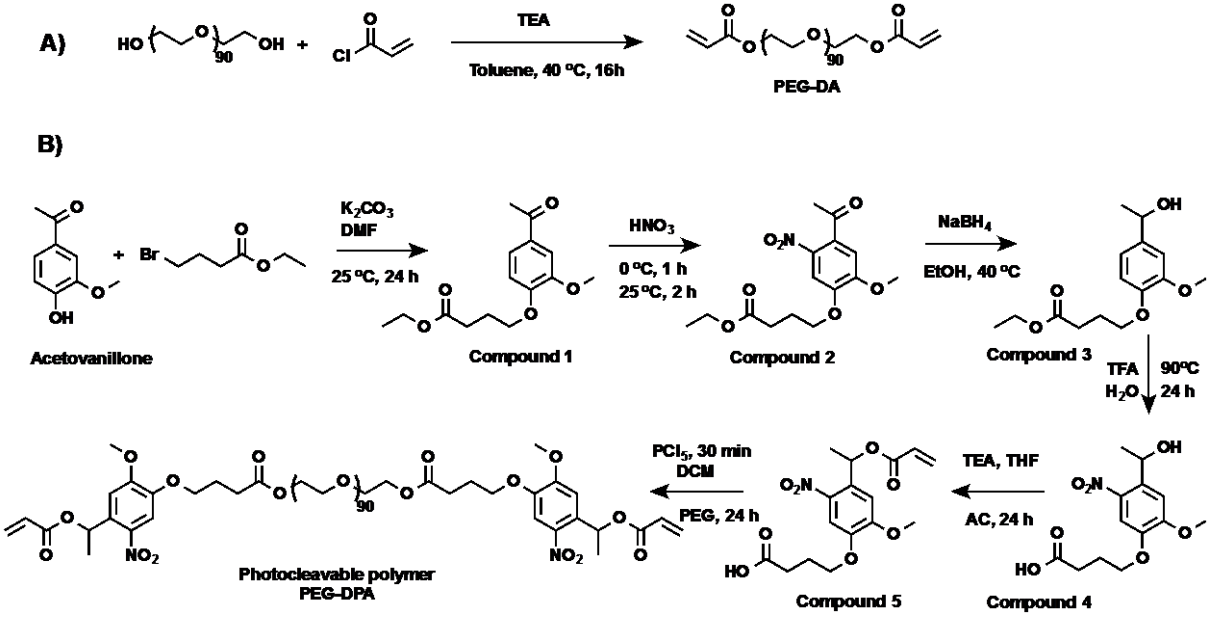
S1.2. Synthesis of poly(ethylene glycol)-diacrylate (PEG-DA)

PEG (Mn 4,000, 8 g) and TEA (1.7 mL) were dissolved in 70 mL toluene. AC (1.06 mL) in 10 mL toluene was added dropwise to the PEG and TEA solution at 0^{o}C. The reaction was performed at 40^{o}C for 16 h, followed by concentrating and precipitating in a 2:1 mixture of ether and hexane to obtain raw polymer powder. To further purify, the polymer was hydrated with deionized water (diH_2O) and dialyzed against diH_2O at 4^{o}C using 3,500 Da cutoff membrane for 3 days. The polymer solution was then frozen and lyophilized until dry.

The conjugation efficiency was 96% as confirmed by ^{1}H NMR (Figure S3A) (Varian Unity-300 (300 MHz) NMR spectrometer, Varian Inc., Palo Alto, CA) [References S1-S3].

S1.3. Synthesis of poly(ethylene glycol)-di(photolabile-acrylate) (PEG-DPA)

PEG-DPA was synthesized according to slightly modified previous reports as shown in Scheme S1 [References S4-S7]. The structure of synthesized compounds was characterized by ^{1}H NMR (Bruker DPX400 Spectrometer, Bruker BioSpin Corp., Billerica, MA).



Scheme S1. Synthesis of (A) PEG-DA and (B) PEG-DPA.

Synthesis of compound 1:

Acetovanillone (10g, 60 mmol), ethyl 4-bromobutyrate (17.6 g, 90 mmol) and potassium carbonate (16.6 g, 120 mmol) were dissolved in DMF (100 mL). The reaction mixture was stirred for 24 h at 25°C, and then precipitated in water (1000 mL). The resultant precipitate was filtered, washed with diH_2O and dried under vacuum to yield 15.6 g compound 1 (93% yield) as a white powder.

^{1}H NMR ($CDCl_3$): δ= 7.52 (m, 2H, Aromatic-**H**), δ=6.88 (d, Aromatic-**H**), δ=4.12 (t, 2H and q, 2H -OC**H$_2$**$CH_2CH_2CO_2$C**H$_2$**CH_3), δ=3.89 (s, 3H, -OC**H$_3$**), δ=2.54 (t, 2H, -

$OCH_2CH_2\mathbf{CH_2}CO_2CH_2CH_3$ and s, 3H, Aromatic-CO$\mathbf{CH_3}$), δ=2.17 (t, 2H, -$OCH_2\mathbf{CH_2}CH_2CO_2CH_2CH_3$), δ=1.24 (t, 3H, -$OCH_2CH_2CH_2CO_2CH_2\mathbf{CH_3}$).

Synthesis of compound 2:

Compound 1 (10 g, 35.7 mmol) was subsequently nitrated with nitric acid (10 mL, 69.9%) at 0°C for 1 h and then allowed to warm to room temperature and react for 2 h. The solution was precipitated in diH_2O and filtered, and the compound 2 was recrystallized from ethanol as a yellow solid and dried under vacuum to yield 8.36 g (72% yield).

^{1}H NMR ($CDCl_3$): δ= 7.61 (s, 1H, Aromatic-**H**), δ=6.74 (s, 1 H, Aromatic-**H**), δ=4.16 (q, 4H, -OC$\mathbf{H_2}CH_2CH_2CO_2C\mathbf{H_2}CH_3$), δ=3.95 (s, 3H, -OC$\mathbf{H_3}$), δ=2.54 (t, 2H, -$OCH_2CH_2\mathbf{CH_2}CO_2CH_2CH_3$), δ=2.49 (s, 3H, Aromatic-CO$\mathbf{CH_3}$), δ=2.2 (m, 2H, -$OCH_2\mathbf{CH_2}CH_2CO_2CH_2CH_3$), δ=1.26 (t, 3H, -$OCH_2CH_2CH_2CO_2CH_2\mathbf{CH_3}$).

Synthesis of compound 3:

Compound 2 (5.1 g, 15.7 mmol) in ethanol was reduced with excess sodium borohydride (1.78 g, 47 mmol) at 40°C. The reaction was stirred 24 h, and compound 3 was precipitated in diH_2O, filtered and dried under vacuum to yield 4.6 g (90% yield).

^{1}H NMR ($CDCl_3$): δ= 7.56 (s, 1H, Aromatic-**H**), δ=7.29 (s, 1 H, Aromatic-**H**), δ=5.55 (m, 1H, -Aromatic$\mathbf{CH}OHCH_3$), δ=4.15 (t, 2H, -OC$\mathbf{H_2}CH_2CH_2CO_2CH_2CH_3$), δ=4.11 (q, 2H, -$OCH_2CH_2CH_2CO_2\mathbf{CH_2}CH_3$), δ=3.97 (s, 3H, -OC$\mathbf{H_3}$), δ=2.53 (t, 2H, -$OCH_2CH_2\mathbf{CH_2}CO_2CH_2CH_3$), δ=2.18 (m, 2H, -$OCH_2\mathbf{CH_2}CH_2CO_2CH_2CH_3$), δ=1.55 (d, 3H, -AromaticCHOH$\mathbf{CH_3}$), δ=1.26 (t, 3H, -$OCH_2CH_2CH_2CO_2CH_2\mathbf{CH_3}$).

Synthesis of compound 4:

The alcohol powder product, compound 3 (2.4 g, 7.34 mmol), was stirred in a solution of trifluoroacetic acid (TFA) (4 mL) and diH_2O (40 mL) at 90°C for 24 h. Additional TFA was added until reaction completion was verified by thin layer chromatography. The reaction

mixture was cooled to 25°C and the precipitate was filtered, washed with chilled diH_2O and dried under vacuum to yield 2.0 g (91% yield).

^{1}H NMR ($CDCl_3$): δ= 7.56 (s, 1H, Aromatic-**H**), δ=7.29 (s, 1 H, Aromatic-**H**), δ=5.56 (q, 1H, -Aromatic**CH**OHCH_3), δ=4.11 (t, 2H, -OC**H$_2$**$CH_2CH_2CO_2H$), δ=3.97 (s, 3H, -OC**H$_3$**), δ=2.55 (t, 2H, -OCH_2CH_2**CH_2**CO_2H), δ=2.18 (m, 2H, -OCH_2**CH_2**CH_2CO_2H), δ=1.55 (d, 3H, -AromaticCHOH**CH_3**).

Synthesis of compound 5:

Compound 4 (2 g, 6.7 mmol) and TEA (3.73 mL, 26.8 mmol) were dissolved in a solution of THF (30 mL) and cooled to 0°C. To this solution, AC (1.9 mL, 23.4 mmol) dissolved in 5 mL THF was added dropwise. The reaction mixture was stirred 24 h at 25°C and then poured into diH_2O (500 mL). The reaction mixture was stirred for 2 h and then extracted with chloroform (100 mL) five times and evaporated to dryness. The monomer was dried under vacuum to yield 2.1 g (89% yield) as a viscous yellow liquid.

^{1}H NMR ($CDCl_3$): δ= 7.58 (s, 1H, Aromatic-**H**), δ=6.99 (s, 1 H, Aromatic-**H**), δ=6.52 (q, 1H, -Aromatic**CH**CH_3O-), δ=6.42, 5.85 (d, d, 2H, Aromatic-$CHCH_3OCOCH$**CH_2**), δ=6.16 (m, 1H, Aromatic-$CHCH_3OCO$**CH**CH_2), δ=4.1 (t, 2H, -OC**H$_2$**$CH_2CH_2CO_2H$), δ=3.92 (s, 3H, -OC**H$_3$**), δ=2.54 (t, 2H, -OCH_2CH_2**CH_2**CO_2H), δ=2.17 (m, 2H, -OCH_2**CH_2**CH_2CO_2H), δ=1.66 (d, 3H, -AromaticCH**CH_3**O-).

Synthesis of PEG-DPA:

Compound 5 (1 g, 2.83 mmol) was reacted with phosphorus pentachloride (1.76 g, 4.81 mmol) for 30 min at 25°C. The resultant phosphorus oxychloride was removed under reduced pressure, and then the residue was dissolved in 20 mL dry DCM. This solution was added dropwise to a solution of PEG (2.8 g, 0.7 mmol) and TEA (0.4 mL, 2.83 mmol) in DCM (20 mL). After stirring 24 h at 25°C, the reaction was filtered and evaporated to dryness. The

product was collected by precipitation in cold diethyl ether (250 mL) and dried under vacuum to yield 2.56g PEG-DPA macromer as a pale-yellow powder (78% yield).

^{1}H NMR ($CDCl_3$): δ= 7.56 (s, 2H, Aromatic-**H**), δ=6.98 (s, 2 H, Aromatic-**H**), δ=6.51 (q, 2 H, -Aromatic**CH**CH_3O-), δ=6.41, 5.85 (d, d, 4 H, Aromatic-$CHCH_3OCOCH$**CH_2**), δ=6.16 (m, 2 H, Aromatic-$CHCH_3OCO$**CH**CH_2), δ=4.23 (t, 4H, Ar-$OCH_2CH_2CH_2CO_2$**CH_2**), δ=4.09 (t, 4H, -O**H_2**$CH_2CH_2CO_2R$), δ=3.9 (s, 6H, -OC**H_3**), δ=3.8-3.48 (m, 360H, PEG), δ=2.55 (t, 4H, -OCH_2CH_2**CH_2**CO_2R), δ=2.16 (m, 4H, -OCH_2**CH_2**CH_2CO_2R), δ=1.64 (d, 6H, -AromaticCH**CH_3**O-). The ^{1}H NMR spectrum of the synthesized PEG-DPA with labeled protons is shown in Figure S3B.

S1.4. Gelation time

The gelation time of the fabricated hydrogels was measured using a protocol we have previous reported [References S1]. Briefly, The TEMED, APS and PEG-DPA or PEG-DA solutions in (total 100 µl at designed hydrogel concentration) were mixed in a 1.7 mL microcentrifuge tube and vortexed for 10s prior to measuring the gelation time. The gelation time is the period from first mixing the material solutions to the point when the mixture stops flowing in the inverted tube (N=3).

S2. Supporting Results

S2.1. Gelation time

Table S1: Gelation time of fabricated hydrogels in this study

Hydrogel	**15% Non-PL**	**15% PL**	**10% PL**	**7.5 % PL**
Gelation time (min), N=3	2.2 ± 0.3	4.8 ± 0.3	8.1 ± 0.4	12.6 ± 0.4

S2.2. UV-triggered siRNA release from photolabile hydrogels formed from different macromer concentrations

Hydrogel concentration is also an important factor that can be used to control the release of bioactive agents from hydrogel systems [References S2, S8, S9]. The capacity to independently control multiple variables that influence siRNA release, such as UV light intensity and exposure duration, and hydrogel concentration, may provide increased flexibility in tuning delivery profiles using this approach. Therefore, the siRNA release kinetics from hydrogels prepared from different concentrations of photolabile macromer (7.5, 10 and 15% w/w) in the absence and presence of "UV 10-20" were performed. The ability to trigger the release of siRNA by UV light exposure can be observed in all experimental hydrogel conditions, as shown in Figure S1. The 7.5% (w/w) hydrogels released siRNA over the course of 11 and 9 days in the absence and presence of "UV 10-20," respectively, which coincided with the complete degradation of hydrogels (Figure S1A). The short siRNA retention time of the "No UV" 7.5% hydrogels resulted from the rapid hydrolytic degradation of ester groups, which were relatively fewer in number due to the lower crosslink density in these hydrogels formed with lower macromer concentration. The "UV" 7.5% hydrogels resulted in even shorter retention due to combined hydrolytic and photolytic degradation mechanisms. A similar trend was observed with the 10 % (w/w) hydrogels with siRNA release occurring over the course of 32 and 17 days for "No UV" and "UV" hydrogels, respectively (Figure S1B). By increasing the hydrogel concentration to 15% (w/w), the release rate of siRNA was decreased further with siRNA coming out of the hydrogels over the course of 44 and 35 days in the absence and presence of "UV 10-20," respectively (Figure S1C). Moreover, while the 7.5% (w/w) hydrogels released all of their encapsulated siRNA when they were completely degraded at day 9 ("UV") and 11 ("No UV"), only 48 and 77% siRNA were released from the "No UV" and "UV" 15% (w/w) hydrogels, respectively, at day 11. These results demonstrate that the release of siRNA is not only triggered by UV light intensity and exposure duration, but it can also be controlled by tailoring hydrogel concentration. Combined tuning of these

system parameters will permit tailorable light-triggered release of siRNA from photolabile hydrogels.

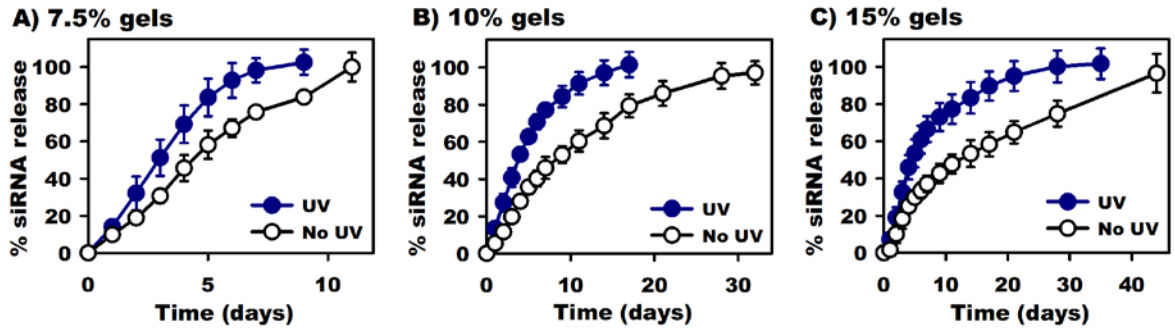


Figure S1. Release profiles of siLuc from (A) 7.5, (B) 10 and (C) 15% (w/w) photolabile hydrogels exposed to "UV 10-20" at each time point.

S2.3. Release of different siRNAs and the effect of UV dose on release kinetics.

At the same hydrogel concentration (15% w/w) and UV dose ("UV 10-20"), the release profile of control siLuc was similar to that of siGFP (Figure S2A). In addition, increasing the UV dose, either by increasing UV exposure time at a constant intensity of 10 mW/cm^2 (Figure S2A) or by increasing UV intensity while keeping a constant UV exposure duration (Figure S2B), can increase the release rate of loaded-siRNA from the photolabile hydrogels.

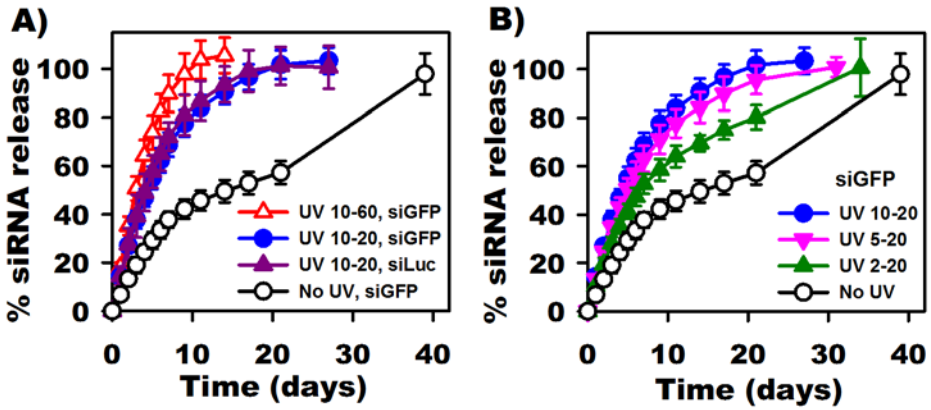


Figure S2. Release profiles of siRNA into DMEM-HG from 15% (w/w) photolabile hydrogels exposed to no UV (containing siGFP) and UV light at (A) an intensity of 10 mW/cm^2 for 20 ("UV 10-20"; containing siGFP or siLuc) and 60 min ("UV 10-60"; containing siGFP) or (B) different intensities of 2, 5 and 10 mW/cm^2 for 20 min ("UV 2-20", "UV 5-20" and "UV 10-20", respectively; containing siGFP).

S2.4. Proton NMR spectra of synthesized PEG-DA and PEG-DPA

^{1}H NMR spectra of the synthesized PEG-DA and PEG-DPA with labeled protons are shown in Figure S3. The acrylation efficiency of PEG-DA, calculated by normalizing the area of acrylate peaks (a) to that of PEG peak (b) in Figure S3A, was 96%. The coupling efficiency of photolabile moiety (compound 5) into PEG, calculated by normalizing the area of acrylate peaks (a) to that of PEG peaks (p) in Figure S3B, was 87.5 %.

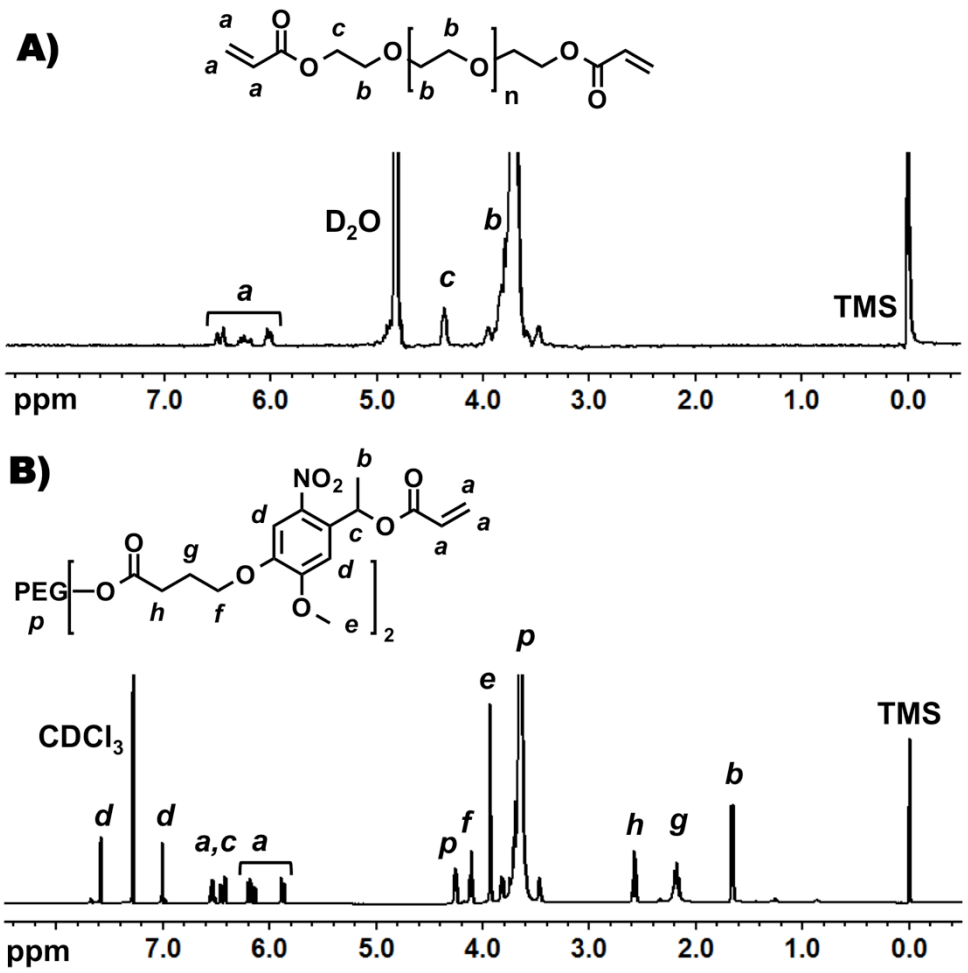


Figure S3. Proton NMR spectra of synthesized (A) PEG-DA and (B) PEG-DPA.

References

[S1]. M. K. Nguyen, O. Jeon, M. D. Krebs, D. Schapira, E. Alsberg, *Biomaterials* **2014**, *35*, 6278.
[S2]. D. P. Huynh, G. J. Im, S. Y. Chae, K. C. Lee, D. S. Lee. *J. Control. Release* **2009**, *137*, 20.
[S3]. C. T. Huynh, S. W. Kang, Y. Li, B. S. Kim, D. S. Lee, *Soft Matter* **2011**, *7*, 8984.
[S4]. G. M. Cruise, D. S. Scharp, J. A. Hubbell, *Biomaterials* **1998**, *19*, 1287.
[S5]. D. Y. Wong, D. R. Griffin, J. Reed, A. M. Kasko, *Macromolecules* **2010**, *43*, 2824.
[S6]. A. M. Kloxin, A. M. Kasko, C. N. Salinas, K. S. Anseth, *Science* **2009**, *324*, 59.

[S7]. D. R. Griffin, A. M. Kasko, *J. Am. Chem. Soc.* **2012**, *134*, 13103.
[S8]. K. Nguyen, P. N. Dang, E. Alsberg, *Acta Biomater.* **2013**, *9*, 4487.
[S9]. C. T. Huynh, Q. V. Nguyen, S. W. Kang, D. S. Lee, *Polymer* **2012**, *53*, 4069.