Supporting Information

Digital maskless photolithographic patterning of DNA-functionalized poly(ethylene glycol) diacrylate hydrogels with visible light enabling photo-directed release of oligonucleotides

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1. Materials and Methods

Materials:

All materials were purchased from commercial vendors and used as received. Poly(ethylene) glycol diacrylate (Mn = 575, 437441) was ordered from Sigma Aldrich and camphorquinone (A14967) and triethanolamine (L04486) were ordered from Alfa Aesar. All oligonucleotides were purchased from Integrated DNA Technologies HPLC purified.

Microfluidic device fabrication:

SU-8 photoresist molds for the flow cell and gradient generator were fabricated on 4-inch silicon wafers with standard contact photolithography. Molds for the gradient generator required two different heights of photoresist which was achieved by first spin coating SU-8 10 (Microchem) to a target height of 20 μm, exposing the wafer to a UV dosage of 225 mJ/cm^2 (i-line), and immersing it in SU-8 developer for 5-10 minutes. After confirming the integrity of the mold of the diffusion cells, a second layer of SU-8 3050 (Microchem) was spin coated over the existing pattern for a target height of 100 μm. Care was taken to make sure that the alignment keys of the first patterned layer weren't covered by the second photoresist layer and these keys were used to align the wafer to the second photomask on a mask aligner. The same exposure dosage and development times described above were used to lift off un-crosslinked photoresist. Poly-dimethyl-siloxane microchannels were made by crosslinking Sylgard 184 (Dow-Corning) in a 10:1 ratio of base elastomer to curing agent. After thermally curing the PDMS mold for 2 hours at 70 °C, devices were cut from the mold, hole punched (3 mm diameter), cleaned in a UV-ozone oven with glass coverslips and annealed together for 2 hours at 80 °C. Photomasks for the branched flow cell and gradient generator are provided as separate CAD documents.

Digital mask design:

Digital masks were generated in AutoCAD and were scaled to fit the areas of the projected micromirror array through a U Plan Fluorite 4X microscope objective.

Multidomain hydrogel formulation:

Two-color hydrogels were fabricated by crosslinking 1 μM fluorescently tagged DNA with a mixture of 75%(v/v) PEGDA 575, 0.8%(w/v) CQ, and 0.5% (v/v) TEA in deionized water. CQ was diluted from a 10% (w/w) stock in 1-butanol. The DNA tag consisted of a 20 bp poly-Thymine strand with a 5' acrydite-modification and a 3' fluorescein or Texas-615 dye modification.

Photopolymerization platform and procedure:

The digital light projection apparatus utilized in our experiments consisted of a Mightex Systems Polygon 400 Dense Pixel micromirror array fitted into the light port of an inverted Olympus IX73 microscope. Blue light was routed from an LED head through a liquid light guide and reflected off of the digital micro-mirror array into the light path of the microscope. We conducted all polymerizations with a 4X microscope objective, which resulted in a maximum exposed rectangular area of 1500 μm in width by 787 μm in height (height = 1.21 μm, width = 2.24 μm per pixel of projected light). We measured the intensity of 470 nm light at the sample stage to be 11.4 mW cm^{-2}. Each digital mask was designed in AutoCAD and rastered onto the digital micromirror array (DMD) through a software interface.

Multi-domain hydrogel procedure: Prior to conducting polyethylene glycol diacrylate (PEGDA) photopolymerization within the branched flow cell, the device was treated with a solution of 12% (v/v) 3-(trimethoxysily)propyl methacrylate in acidic methanol to functionalize the channel surface with pendant methacrylate groups in order anchor the hydrogel within the channels. Tygon tubing (Cole Parmer - 0.060 in. OD) was inserted into its inlet and outlet, and the outlet tube was placed into a 50 mL Falcon tube to collect effluent. Solutions were injected manually into the device using a 1mL syringe (Becton Dickinson). The 75% (v/v) PEGDA-DNA prepolymer formulation consisted of 75% (v/v) PEGDA (Sigma Aldrich), 0.8% (w/v) (±) camphorquinone (CQ), and 0.5% (v/v) triethanolamine (TEA) (Alfa Aesar). To promote its solubility in the final pregel blend, CQ was first dissolved in a 10% (w/w) solution of 1-butanol and diluted down to a final 0.8% (w/v) in the pregel solution. The device was placed on the microscope stage and prepolymer solutions were injected serially into the channels. Exposure to patterns of blue light were 5 seconds long. After each set of patterns was generated, water was injected into the device manually for several minutes; the syringe was then replaced with a new syringe holding the second DNA pregel solution, and the injection and exposure process was repeated a second time. Images were obtained using time-lapse fluorescence microscopy using a 16-bit Infinity 3 CCD camera.

Gradient generation procedure: The microfluidic network utilized in the diffusion experiments consisted of up to 25 diffusion cells lined on each side by a liquid delivery channel; each diffusion cell was 1500 μm long, 50 μm in width, and 20 μm in height. Cells were grouped into arrays of 5 channels with an inter-channel spacing of 50 μm; we designed each array of cells to fight exactly within the projected area of the entire array of exposed mirrors. The gradient generator was first methacrylated with 12% (v/v) 3-(trimethoxysily)propyl methacrylate in acidic methanol as previously described before we conducted experiments. The prepolymer blend utilized in the gradient experiments consisted of 30% (v/v) PEGDA 575, 0.5% (v/v) TEA, 0.8% (w/v) CQ, and 1X tris-acetate-EDTA (TAE) buffer with 12.5 mM Mg^{2+}. To produce a gradient of DNA within the patterned substrates, a 30% (v/v) PEGDA prepolymer solution was first injected into the diffusion cells manually and exposed to the full array projected from the 4X objective for a dosage of 228 mJ cm^{-2}. The polymerization was conducted for 20 s on each array of 5 microchannels in the gradient generator (5 total arrays per device). Tygon tubes were then connected to the device inlet and outlet ports and a fluidic controller[1] supplied constant pressure-driven flow of a DNA oligonucleotide and non-DNA solution (both contained 1X TAE Mg^{2+} buffer) to opposite sides of the diffusion cells; liquid was routed through a resistor upstream of the diffusion cells to mitigate convection due to minute pressure imbalances on the two side of the diffusion channels. Images were obtained using time-lapse fluorescence microscopy using a 16-bit Infinity 3 CCD camera at 400 ms exposure. Dark frame correction was performed on individual images to remove artifacts from uneven signal intensity across the CCD array. We constructed montages of the microchannel arrays by digitally appending individual images with overlapping fields of view and smoothing of the montage to remove residual noise.

Crosslinking Efficiency & Photocleavage of DNA within hydrogels: DNA photocleavage experiments in the hydrogels were conducted in the methacrylated branched flow cells using the 75% PEGDA formulation. To determine the crosslinking efficiency of CQ and TEA with acrydite modified DNA, 2 μM of Anchor 1 was mixed into the pregel solution and injected into the microfluidic device. 750 μm diameter circles were patterned in the flow cell at a dosage of 57 mJ cm^{-2}. To quantify how much DNA was crosslinked during photopolymerization, a New Era NE-500 syringe pump holding a 20 mL syringe of water was connected to the flow cell with Tygon tubing and the gels were washed within the channels with for several hours and imaged over time using a 10X UPlan FL N microscope objective. The reduction of the fluorescence intensity profile in the hydrogel was normalized by the intensity profile obtained immediately after photopolymerization and before the washing step (**Figure S4)**. Roughly 12% of the DNA was retained after the wash step. An identical experimental set up was used to examine UV-triggered cleavage and release of Anchor 2 from 75% PEDGA hydrogels. 750 μm diameter circles were patterned in the flow cell at a blue light dosage of 57 mJ cm^{-2} and were washed with water for 24 hours using a syringe pump. After UV exposure, 2 μM of Probe 2 in 1X TAE Mg^{2+} buffer was then washed into the flow cells and allowed to diffuse into the hydrogels for 24 hours, the flow cell was then washed with 1X TAE Mg^{2+} buffer for another 5 hours and the gels were imaged.

Synthetic Oligonucleotides:

The sequences of the DNA strands utilized in this study are listed below:
Multi-domain DNA Hydrogels:
/5Acryd/TTTTTTTTTTTTTTTTTTT/36-FAM/

/5Acryd/TTTTTTTTTTTTTTTTTTT/3TEX615/

Diffusion Gradient (Probe 1):
/5Cy3/TCTACGGAAATGTGGCAGAATCAATCATAAGACACCAGTCGG

Simple Diffusion Experiment (Probe 2):
/5Cy3/CATCTCATAACACATCTCACAATCCATCTCA

Acrydite-DNA anchoring and photocleavage experiments:
Anchor 1: /5Acryd/TTTTT/iSpPC/TTTTT/3Cy3Sp/
Anchor 2: /5Acryd/TTT/iSpPC/TGAGATGGATTGTGAGATGT

Camphorquinone/1-(2-nitrophenyl)ethyl linker compatibility:
S1_PC_S4: TCCATTCCACT/iSpPC/CATAACAACCA
S4'_PC_S1': TGGTTGTTATG/iSpPC/AGTGGAATGGA

Characterization of DNA diffusivity in 30% PEGDA hydrogels:

Within the channels of the microfluidic gradient generator, if different concentrations of a DNA strand exist at opposite ends of the hydrogel, a linear gradient should form at steady state, assuming homogenous diffusion. In order to determine the diffusion coefficient of the DNA probe in the hydrogel, we treated the dynamics of diffusion within the channel as a one-dimensional diffusion process. The steady state solution to the 1-D diffusion equation with Dirichlet boundary conditions is a line:

$$\frac{\partial C(x,t)}{\partial t} = D\nabla^2 C(x,t) \quad (1)$$

$$C(x,t)|_{x=0} = 0 \quad (2)$$

$$C(x,t)|_{x=L} = C_0 \quad (3)$$

$$\frac{C(x,t)}{C_0} = \frac{x}{L} + 2\sum_{i=1}^{\infty}\left(\frac{i\pi\cos i\pi - \sin i\pi}{i^2\pi^2}\right)\sin\left(\frac{i\pi x}{L}\right)e^{-(i\pi)^2 t\frac{D}{L^2}} \quad (4)$$

In this 1-D diffusion process, a linear gradient exists at steady state only if there is no convection and isotropic diffusion across the channels. The concentrations at the left-hand-side and right-hand-side boundaries of the channels were 0 and 200 nM of Probe 1. In our experiments, we observed that linear gradients formed in the channels and remained stable during the entire time course of the experiment, suggesting that these conditions were met. We used least-squares fitting of fluorescent count data to the solution of the 1-D diffusion equation for our specific boundary conditions to calculate the diffusion coefficient of the oligonucleotide. The solution is the superposition of time-independent and time-decaying solutions (Eqn. 4).

Characterization of DNA diffusivity in 75% PEGDA hydrogels:

To determine the diffusivity of a 31-base long oligonucleotide in the 75% (v/v) PEGDA formulation, we first polymerized 500 μm diameter circles in the branched flow cell for dosages of 22, 34, 47, and 57 mJ cm^{-2}. A solution of 2 μM of Probe 2 in water was injected into the device for roughly 2 minutes. The device was then time lapse imaged to measure the rate of diffusion of the DNA into the hydrogels. In order to calculate the diffusion coefficient of the DNA, intensity profiles of the DNA were least-squares fit to the solution to the diffusion equation in cylindrical coordinates (eqn. 9) with the following boundary and initial conditions:

$$\frac{\partial C(r,t)}{\partial t} = D\nabla^2 C(r,t) \quad (5)$$

$$\left.\frac{\partial C(r,t)}{\partial r}\right|_{r=0} = 0 \quad (6)$$

$$C(r,t)|_{r=R} = C_0 \quad (7)$$

$$C(r,t)|_{0<r<R,\ t=0} = 0 \quad (8)$$

$$\frac{C(r,t)}{C_0} = 1 - \sum_{n=1}^{\infty} \left(\frac{J_1(\lambda_n)}{\frac{\lambda_n^2}{2}(J_o^2(\lambda_n) + J_1^2(\lambda_n))} \right) J_o\left(\frac{r}{R}\right) e^{-\lambda^2 t \frac{D}{R^2}} \quad (9)$$

where are J_o and J_1 Bessel functions of order 0 and 1 of the first kind respectively. Graphs of the normalized intensity profile in the hydrogels over the dosage range tested are listed in Figure S3.

2. Supplemental Data

2.1 Camphorquinone compatibility with a photocleavable oligonucleotide 1-(2-nitrophenyl)ethyl spacer:

We verified that the presence of camphorquinone does not inhibit UV-triggered photocleavage of an internally placed 1-(2-nitrophenyl)ethyl linker in a 22 bp DNA duplex. The specific sequence of the duplex (consisting of strands S1_PC_S4 and S4'_PC_S1') was adapted from a library of domains provided in Qian and Winfree 2011[2]. We hybridized the DNA duplex by mixing equimolar amounts of each strand with 1X TAE Mg^{2+} buffer in an Eppendorf Mastercycler PCR; the anneal protocol consisted of heating the solution up to 90 °C for 5 minutes and then cooling 1 °C every minute to 20 °C. The position of the PC spacer is the same on each strand, so that photocleavage should result in the scission of the duplex into two 11-bp fragments, which exhibit a different electrophoretic mobility than the full duplex.[3–6] 1 μM of the DNA duplex was mixed with 1% (v/v) Irgacure 2100 (BASF), a UV photoinitiator with peak absorbances at 275 and 370 nm, or 0.8% (w/v) CQ and exposed to 1, 5, and 10 minutes of 302 nm radiation from a UVP benchtop transilluminator. DNA from the exposed solutions was separated using polyacrylamide gel electrophoresis (PAGE) with a 10% polyacrylamide gel run at 120 V for 1.5 hours. PAGE gels were stained with Sybr Gold (ThermoFisher), a DNA intercalating dye, and visualized on a gel imager. A 100 bp double stranded DNA ladder (ThermoFisher) was used to track the size-dependent separation of the DNA fragments. The presence of 1% (v/v) Irgacure 2100 significantly diminished photo-scission of the duplex across all exposure times. However, the duplex exhibited cleavage at all exposure times when mixed with 0.8% (w/v) camphorquinone.

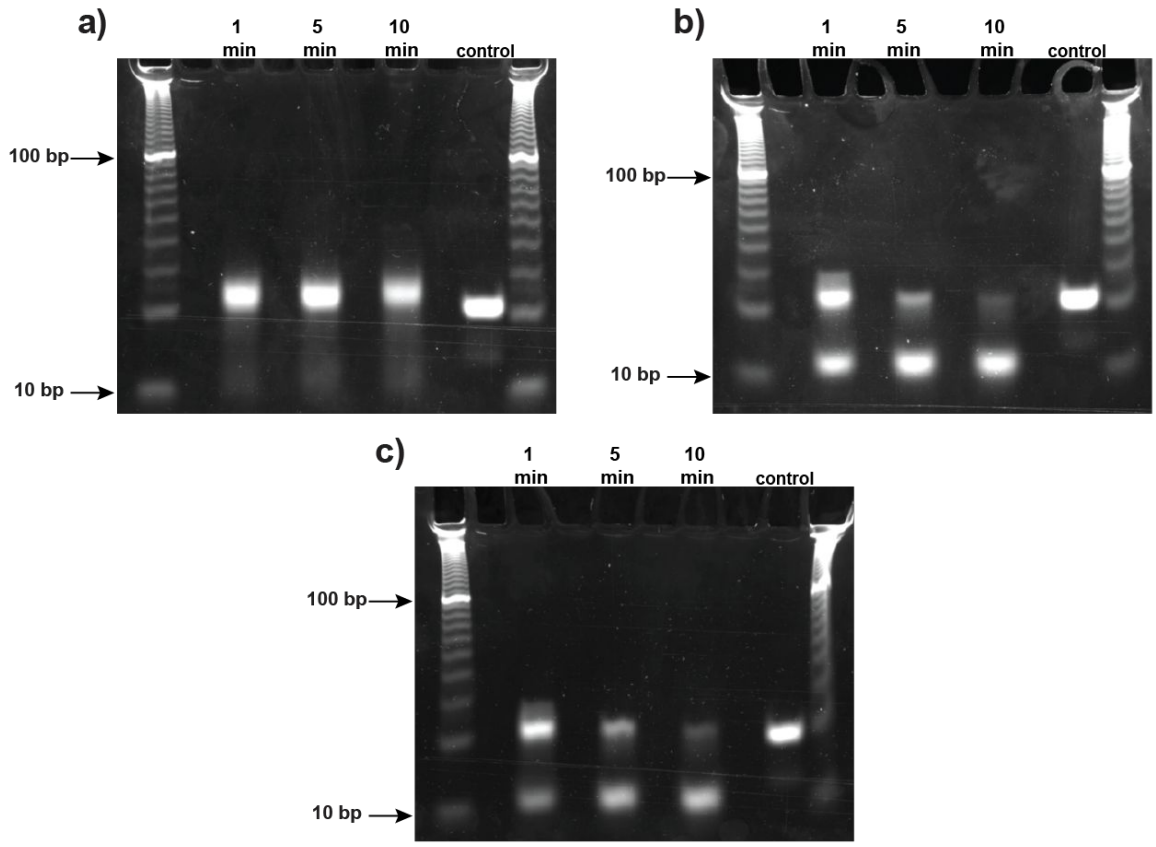

Figure S1. DNA duplex PC-linker cleavage in the presence of a) 1% (v/v) OmniRad/Irgacure 2100 b) 0.8% (w/v) CQ, and c) 1X TAE Mg^{2+} buffer.

2.2 Fluorophore compatibility with UV light exposure:

We identified Cyanine 3 as a DNA dye modification that exhibited minimal photobleaching when exposed to UV-A light. To confirm its behavior, we first exposed solutions of 1 μM Cyanine 3 labeled DNA in 1X TAE Mg^{2+} buffer to UV-A light emitted from a UVP benchtop transilluminator for 2 hours and observed a 5% average change in the average fluorescence intensity of the solution over that period of time (Figure S2). To determine whether camphorquinone potentially degraded Cy3 dye fluorescence activity during excitation under UV light, we also exposed solutions of Cyanine 3 labeled DNA mixed with 0.8 % (w/v) CQ for 2 hours and observed an average reduction in fluorescence intensity of 38% (Figure S2). Having established that CQ and Cy3 had a deleterious interaction during exposure to UV light, we visualized the UV-directed release of DNA from CQ photopolymerized hydrogels (see main text) by allowing a Cy3-modified strand to diffuse into the gels and hybridize to its crosslinked photocleavable reverse complement several hours after UV exposure.

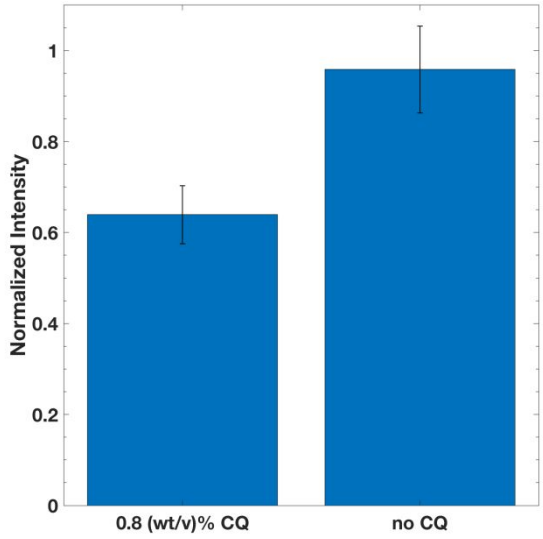

Figure S2. Fluorescence intensities of solutions of Cy3-labeled DNA exposed 2 hours of UV-A radiation on a UVP benchtop transilluminator in the presence and absence of CQ (mean ± s.d.). Normalized intensity is intensity as compared to initial intensity before UV exposure.

2.3 Estimation of DNA diffusion coefficients within 75%(v/v) PEGDA hydrogels:

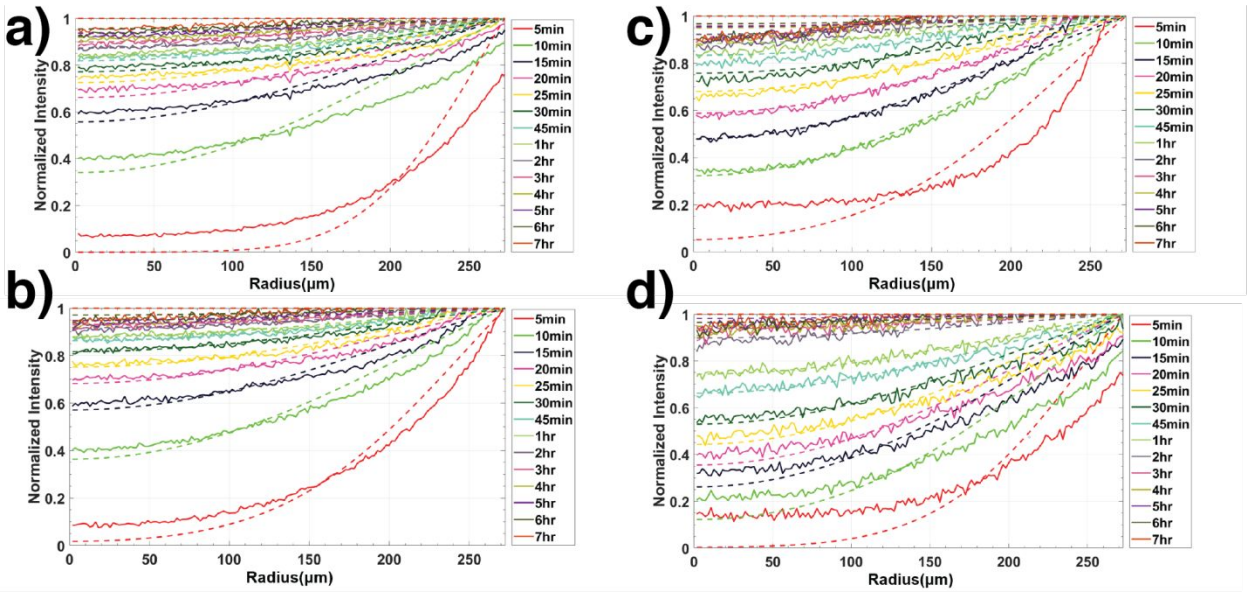

Figure S3. Graphs of radial fluorescence intensity profiles of Probe 2 (see Materials and Methods: Synthetic Oligonucleotides for sequence) diffusing into 500 μm diameter 75% (v/v) PEGDA hydrogels photopolymerized for dosages of (a) 22 mJ cm^{-2} (b) 34 mJ cm^{-2} (c) 47 mJ cm^{-2} (d) 57 mJ cm^{-2}.

2.4 Notes on the uniformity of hydrogel height resulting from digital photopolymerization process:

While we did not characterize the uniformity of DNA diffusivity with respect to hydrogel height or z-dimension, we expect that DNA also diffuses uniformly through the hydrogels in this dimension as well. The physics of the decay of light intensity across the z-depth are well-studied in the context of digital light projection photolithography[7,8], for bulk photopolymerization of macroscope volumes of acrylate resins with CQ[9], and obey the Beer-Lampert law. We estimate that the ratio of the intensity of

470 nm light at a depth of z in our devices to its incident intensity (I_z/I_o) drops to .97 and .87 for depths of 20 μm and 100 μm respectively.[10] A 13% drop in light intensity may result in variations in the microstructure of the hydrogel, specifically with its pore size. However, keeping the CQ photoinitiator concentration below 1% mass of the prepolymer solution is known to mitigate its screening effects across the polymerized depth, which was the case for the formulations presented in this work.[9]

2.5 System factors impacting digital photocleavage efficiency within PEGDA-*co*-DNA hydrogels:

The efficiency of the photocleavage reaction is highly dependent on the reflectivity of the micromirrors and transmittance of the microscope projection optics; certain aspects of our projection system were not optimized for UV transmission. The extinction coefficient of the 1-(2-nitrophenyl)ethyl linker is optimized to absorb and cleave radiation below 350 nm. At 365 nm and above, the moiety's quantum yield drops significantly. While the UV mercury lamp we used for photocleavage emitted lines from 320 nm to 400 nm, the mirror set of the DMD only transmits light at 350 nm and above. Moreover, the transmission of the multipurpose 4X UPlan Fluorite objective used in our study cuts off around 350 nm and has a transmittance of 50% at 365 nm.[7] Our system is highly inefficient for transmitting UV light onto the hydrogel substrate. As such, it is not surprising that the photocleavage efficiency we measured over the range of exposure dosages was low. The quality of the moiety incorporated during DNA synthesis by the manufacturer (Integrated DNA Technologies) may also play a role in its cleavage efficiency, defective or non-reactive moieties will contribute to the overall efficiency observed. It is also important to note that unlike the blue-light photopolymerization process which occurred in seconds of exposure time, during the photocleavage exposure, UV light travels through densely crosslinked polymer across the entire microchannel thickness; attenuation of UV light intensity at increasing depth due to scattering and absorption by the crosslinked PEGDA macromer[11,12] could also diminish the ability to cleave the 1-(2-nitrophenyl)ethyl linker.

2.6 DNA Crosslinking Efficiency of CQ/TEA in 75% (v/v) PEGDA:

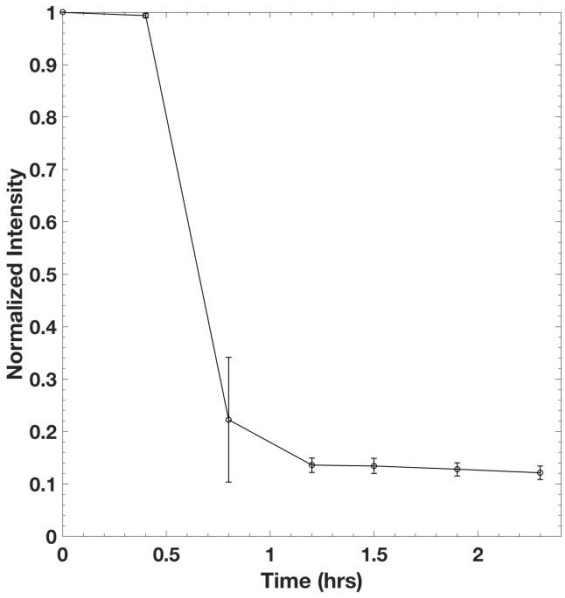

Figure S4. Fraction of Anchor 1 retained during washing of 75% (v/v) PEGDA hydrogels, average center intensity (mean ± s.d.) for 11 gels.

2.7 PEGDA-DNA hydrogel crosslinking schematic:

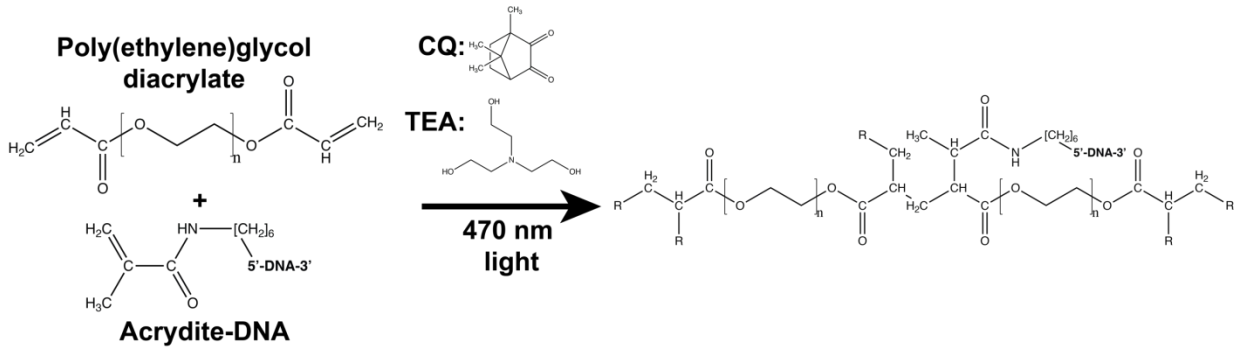

Figure S5. Co-photopolymerization of PEGDA and 5' acrylate-modified DNA.

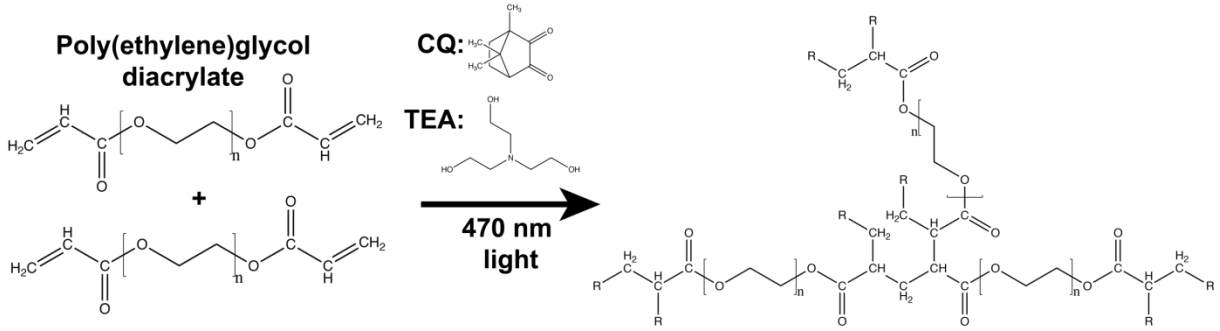

Figure S6. PEGDA macromer photopolymerization.

2.8 UV photoscission of 1-(2-nitrophenyl)ethyl linker schematic:

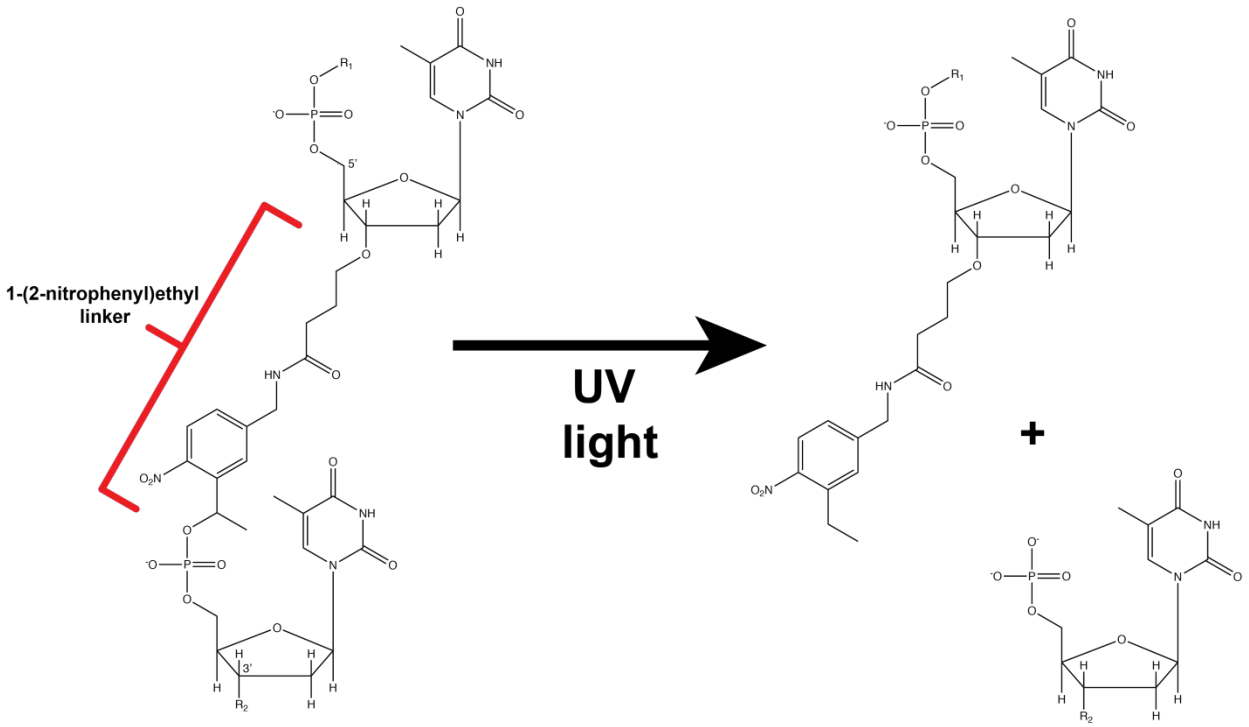

Figure S7. UV triggered photocleavage of a 1-(2-nitrophenyl)ethyl linker inserted in the backbone between two Thymine bases.

References:

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