Supporting Information

Photo-responsive Photonic Hydrogel: in-situ Manipulating and Monitoring Cell Scaffold Stiffness

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Experimental Section

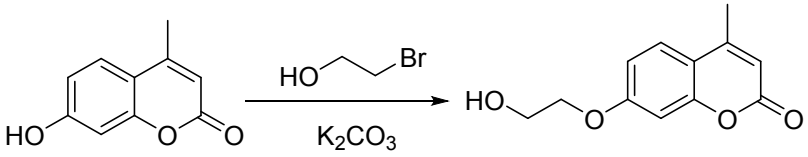
Materials.

Acrylamide (AAm) and poly (ethylene glycol) diacrylate 700 (PEGDA 700) were obtained from Sigma-Aldrich. 4-Methylumbelliferone, 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (photo initiator 2959), Rhodamine B, N, N'-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), polyethylene glycol monoester methacrylate (PEGMA), 2-bromoethanol, quartz sand and acryloyl chloride were obtained from Aladdin (China). Dimethyl sulfoxide (DMSO), triethylamine, tetrahydrofuran (THF), dichloromethane (DCM), petroleum ether, ethyl acetate and other solvents were obtained from Macklin (China). Silica nanospheres were obtained from Nano rainbow (China).

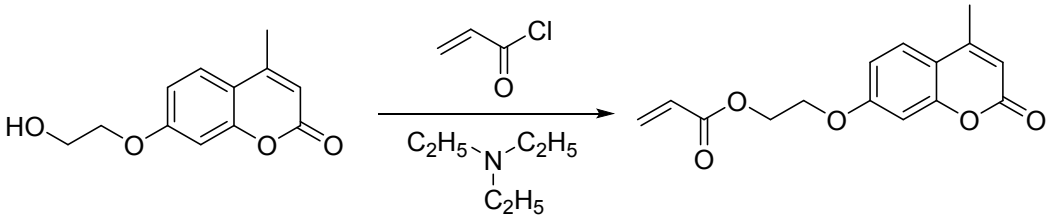
The glass plates used in the experiments were 7101 microscope slides from Sail Brand (China). A GY-6-point light source fitted with a 200 W Hg lamp (Tian Jin Tuo Pu Instruments Co., Ltd, China) was utilized for the photopatterning process. The intensity of the irradiated UV light was 5 mW/cm^2. For other UV illumination experiments, UVP CL-1000L (λ=365 nm, Analytik Jena US, Ltd, USA) and UVP CL-1000 (λ=254 nm, Analytik Jena US, Ltd, USA) crosslinkers were used, respectively.

7-(2-hydroxyethoxy)-4-methyl-coumarin: 7-hydroxy-4-methylcoumarin (1.00 g, 5.71 mmol) was dissolved in 40 mL DMSO. K_2CO_3 (0.60 g, 4.35 mmol) was added, and then 2-bromoethanol (1.00 g, 8.00 mmol) was added dropwise. The solution was stirred at 90 ^oC for 20 h, cooled to room temperature, and slowly introduced into 4 volumes of ice water, leading

to a pale-yellow precipitate. The precipitate was then filtered off and desiccated (90% yield). The crude product was directly used in the next step without further purification. The structure of the product was confirmed by ^{1}H NMR (Figure S1).



7-(acryloyloxy ethoxy)-4-methyl-coumarin (coumarin-containing monomer, CA monomer): 7-(2-hydroxyethoxy)-4-methyl-coumarin (1.00 g, 4.54 mmol) was dissolved in 40 mL of DMSO containing triethylamine (1.50 mL, 10.79 mmol), and acryloyl chloride (1.00 mL, 12.3 mmol) was added dropwisely. The reaction was protected from light for 2 hours at room temperature. Then the solution was poured into 200 mL of deionized water, and the precipitate was obtained by filtration. The crude product was further purified by column chromatography (n-hexane: ethyl acetate = 5:1), and the obtained solid was dried in vacuum for 24 hours (0.647 g, 73%) to give 7-(acryloyloxy ethoxy)-4-methyl-coumarin. The structure of the product was confirmed by ^{1}H NMR test (Figure S2).



Formation of coumarin-containing hydrogel. AAm (20.00 mg), CA monomer (3.00 mg), PEGDA 700 (1 μL), and photo initiator 2959 (0.30 mg) were added into DMSO (90 μL). After 5 min of ultrasound treatment, the solution was allowed to infiltrate into a gap between two parallel glasses separated by a polyimide spacer (75 μm thickness) and then exposed to UV light (λ = 365 nm) to photopolymerize the monomers (①-④). Since CA monomer is difficult to be dissolved in water, DMSO was used as the solvent in the fabrication process. After UV polymerization, the obtained gel was immersed in water for several hours to replace DMSO

with water, leading to the final hydrogel (⑤-⑥). A poly (AAm-PEGDA-CA) hydrogel film was thereby obtained.

Formation of photonic hydrogel. AAm (20.00 mg), CA monomer (3.00 mg), PEGDA 700 (1 μL) and photo initiator 2959 (0.30 mg) were added into DMSO-dispersed silica particles (120 μL, Φ152 nm 60% (w/v)). The mixture was then polymerized with the same process described above. Although the concentration of silica is higher than previously reported photonic hydrogels (5~15%), the obtained hydrogel is still very soft (Movie S1). Some results from the nanoindentation test were provided (Figure S7).

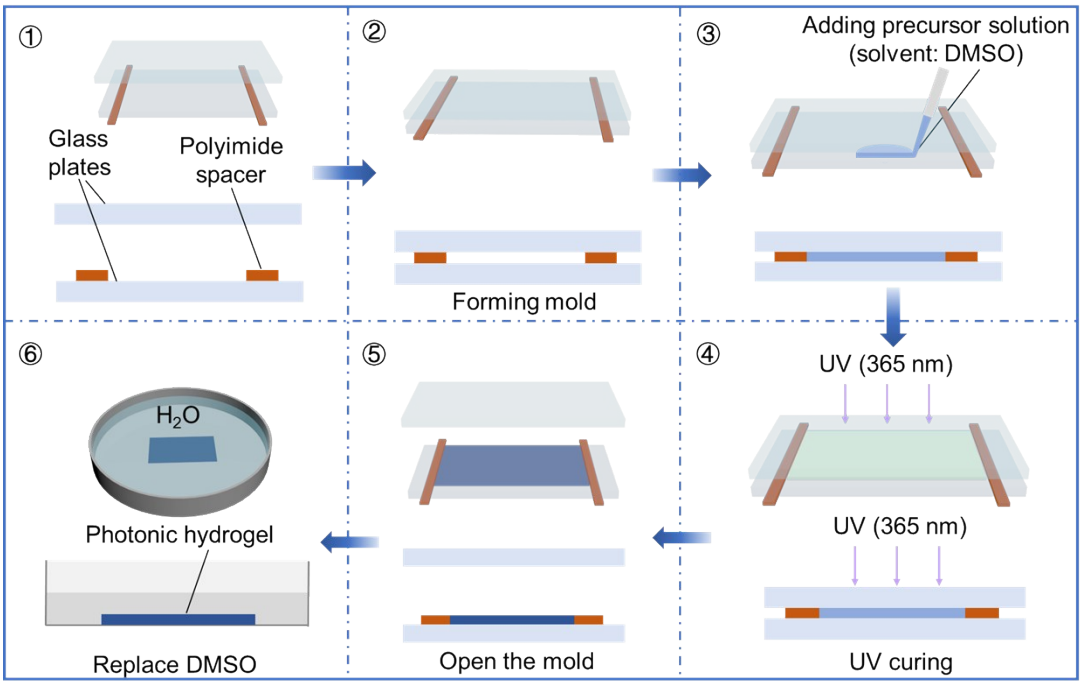


Photo-reconfiguration of hydrogel

Mechanical reconfiguration. The prepared hydrogel film was placed on a glass slide and exposed to UV light (254 nm) for 2 min, 4 min, and 5 min respectively, to de-crosslink the polymer network and form hydrogels of different stiffness and colors.

Chemical reconfiguration. The coumarin-containing hydrogel was de-dimerized with UV light (254 nm) for 5 min, to form coumarin groups in the hydrogel. Then the hydrogel was immersed in RLC solution (5 mg/mL in DMSO) for 6 h, and irradiated with UV light (365 nm)

for 5 min. The obtained hydrogel was then immersed in DMSO overnight to remove unreacted RLC.

Photopatterning. The photopatterning process is similar to the mechanical reconfiguration process described above (UV time: 5 min), a photomask was applied during irradiation to obtain corresponding patterns.

Cell culture on hydrogels. Fibroblasts were routinely cultured in DMEM containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin on tissue culture-grade polystyrene at 37 °C in a 5% CO_2 atmosphere. To increase cell affinity, gelatin was grafted onto the surface of the hydrogel according to a previously described procedure (Journal of Visualized Experiment 2015, **97**, 52643,). The hydrogel film was then sterilized in 75% ethanol for 3 hours, in sterile PBS for at least 3 hours and in cell culture medium for 1 hour. The cell culture medium was removed, and a new cell culture medium was injected. Subsequently, the pretreated cells were suspended on the surface of the hydrogel at a concentration of 2×10^5 cells/mL and were observed by microscopy and imaged after 24 hours of incubation.

Extraction of color information from photonic hydrogels

The images of the photonic hydrogels were taken by a Sony ILCE-6300 mirrorless digital camera equipped with a Sony SEL 30mm f/3.5 e-mount macro fixed lens. All images were obtained under same conditions (aperture value, shutter time, ISO, at a fix position under a while light source). To ensure that the white balance of all images was correct, a grey card was placed beside the hydrogel during the photographing, and the white balance of the obtained images were calibrated in Adobe Photoshop using the white balance pipette function.

After pretreatment, the color space of the images was switched from RGB to HSV by RGB2HSV function in MATLAB. Then the hue map of the image could be extract directly. A technical problem will occur in this process, that on the hue circle, the red color arranges on the

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both sides of 0°/360 ° point, this makes the red region on the hue map highly noisy. To solve this problem, a filter was applied on the obtained hue map to remove all data points with value large than 220 (hue value of original blue color) and replace these values with 360 -X (X refers to the hue value of corresponding data point) to transfer all the red points on the right side of 0°/360 ° point to the left side.

The obtained hue map of the photonic hydrogel contains some noise points due to the misidentification of the colors by the camera during photographing. Therefore, a filloutliers process (in MATLAB) was applied to remove these noises. To do this, filloutliers function was used to find out the outliers in the image, which were then substituted with Neighboring non-outliers by "nearest" method. Fspecial function was designated to create a 3×3 Smoothing filter template, leading to a smoothed graph.

Calculating stiffness values from hue values (color fitting process)

By extracting the hue value of the hydrogel in Figure 3c, it is possible to establish the relationship between the hue and stiffness of photonic hydrogel (Figure S5). An equation could be fitted in Origin 8.0 software using the data in Figure S5 (notably, the equation only works for hydrogel with corresponding composition). By this equation, all hue values in the hue map could be converted to stiffness values using MATLAB, therefore a hue map could be directly transferred to a stiffness map.

UV-Vis spectrometry. The UV-Vis spectrum of the hydrogels was measured using a UV-Vis spectrophotometer (UV-6100, MAPADA, China).

Reflectance spectrometry. The reflection and fluorescence spectra of the hydrogels were recorded by an optical microscope equipped with a fiber-optic spectrometer (Ocean Optics, QE65000).

^{1}H NMR. The CA monomer was analyzed using nuclear magnetic resonance spectroscopy (Bruker DRX-500, Switzerland). The solid was dissolved in deuterated chloroform and diluted to 0.05% before analysis.

SEM test. SEM images were obtained using a field emission scanning electron microscope (Zeiss Ultra Plus, Germany). The samples were sputtered with 30 nm gold layer using a Hitachi E-1010 ion sputter (Hitachi, Ltd., Japan) before measurement.

Nanoindentation. The mechanical distribution of the hydrogels was obtained using a nanoindenter (Piuma, Netherlands). A 0.05 μm probe was used.

Supporting figures

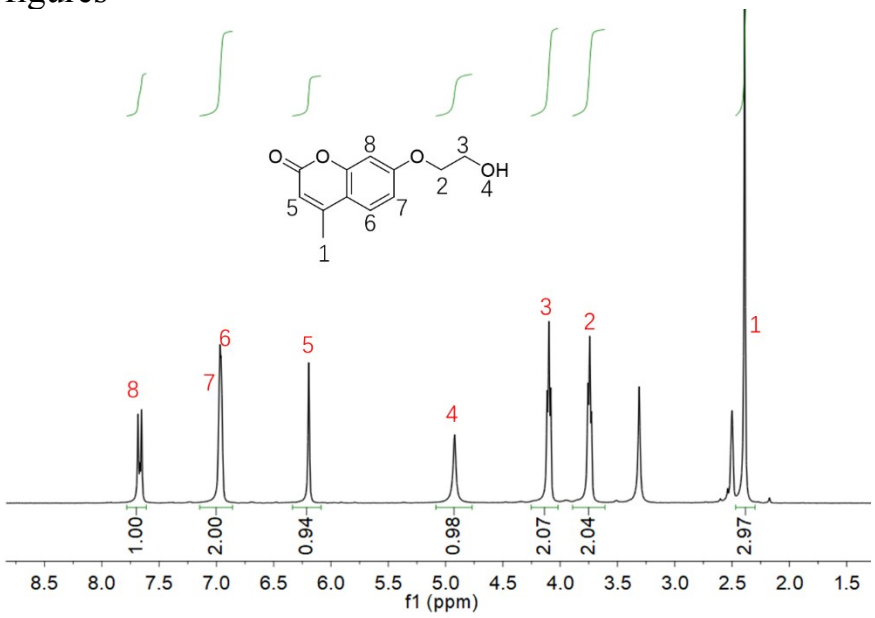

Figure S1. ^{1}H nuclear magnetic resonance spectra (^{1}H NMR) obtained from the 7-(2-hydroxyethoxy)-4-methyl-coumarin.

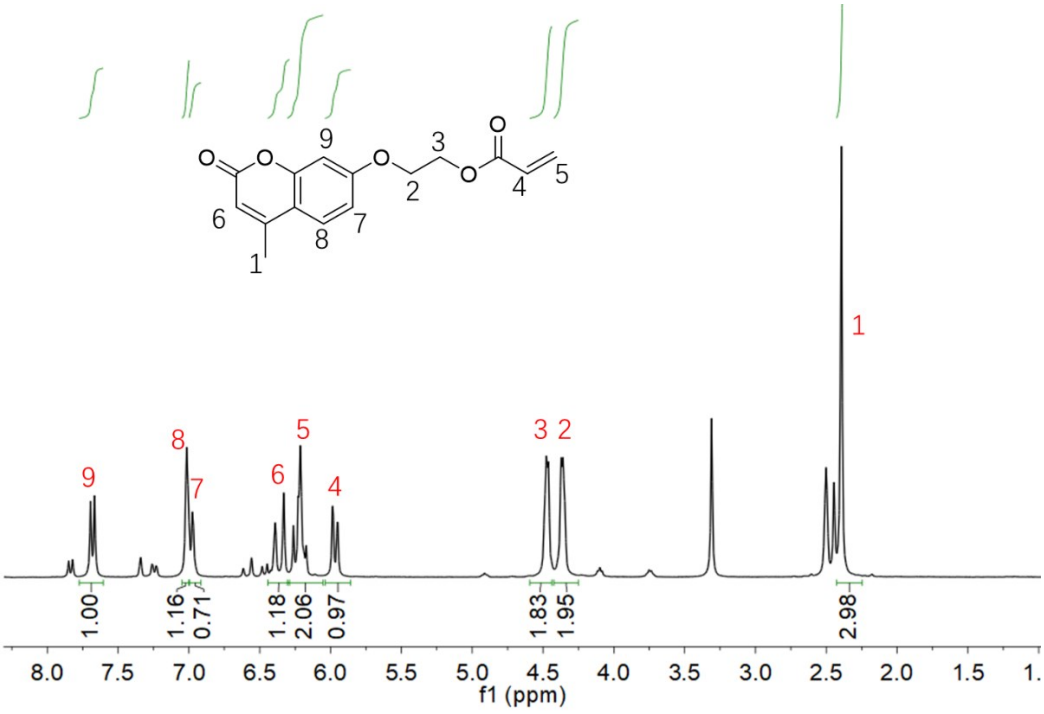

Figure S2. ^{1}H nuclear magnetic resonance spectra (^{1}H NMR) obtained from the 7-(acryloyloxy ethoxy)-4-methyl-coumarin.

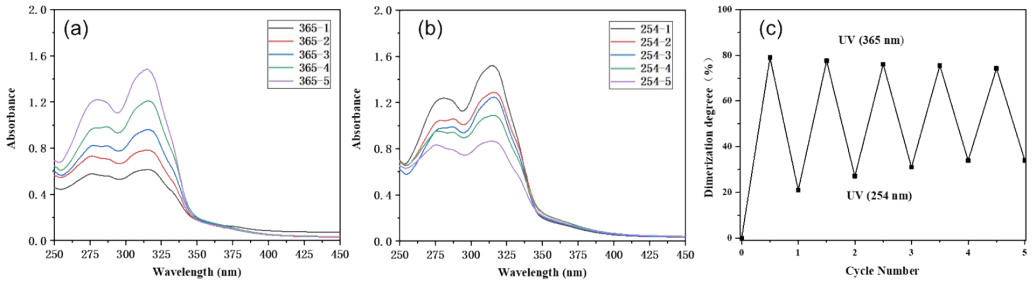

Figure S3. The UV-Vis absorption spectra of the coumarin-containing hydrogel after alternate exposure to (a) 365 nm and (b) 254 nm UV light. (c) Reversibility in the degree of dimerization of the hydrogel during five cycles of UV irradiation with 365 nm and 254 nm. The tests were performed according to a previous report (*Chem. Mater.* **2016**, *28*, 6401).

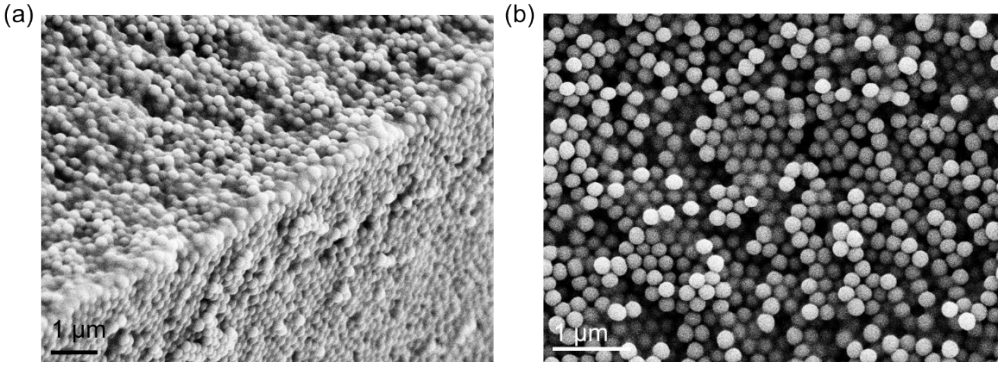

Figure S4. SEM images of the freeze-dried colloidal crystal hydrogel interface. (a) A photograph showing the cross-section of the hydrogel that was taken using a 45° tilt of the sample stage. (b) A photograph showing a cross-section of the hydrogel. Since the hydrogel is lyophilized, the hydrogel shrinks after dehydration and is thus invisible from the top view.

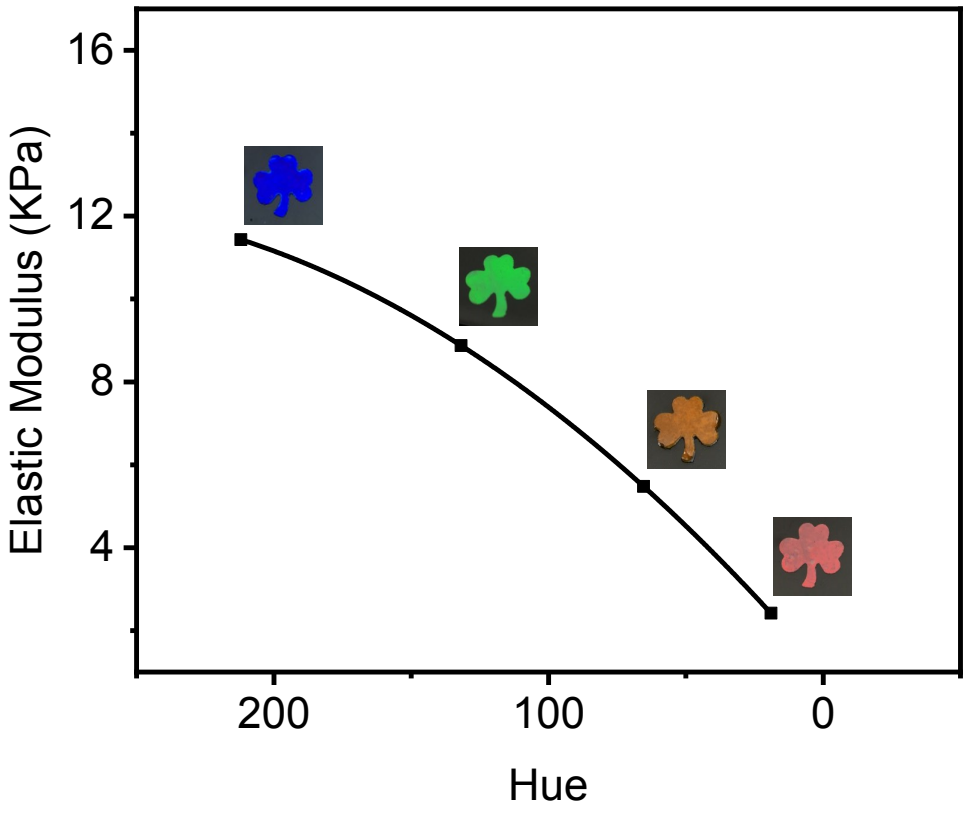

Figure S5. Relationship between hue value of the apparent color and stiffness of the photonic hydrogel. The figure is obtained by extracting the hue value of the photonic hydrogel from the image (as X axis) and use the stiffness value obtained from nano indentation as Y axis.

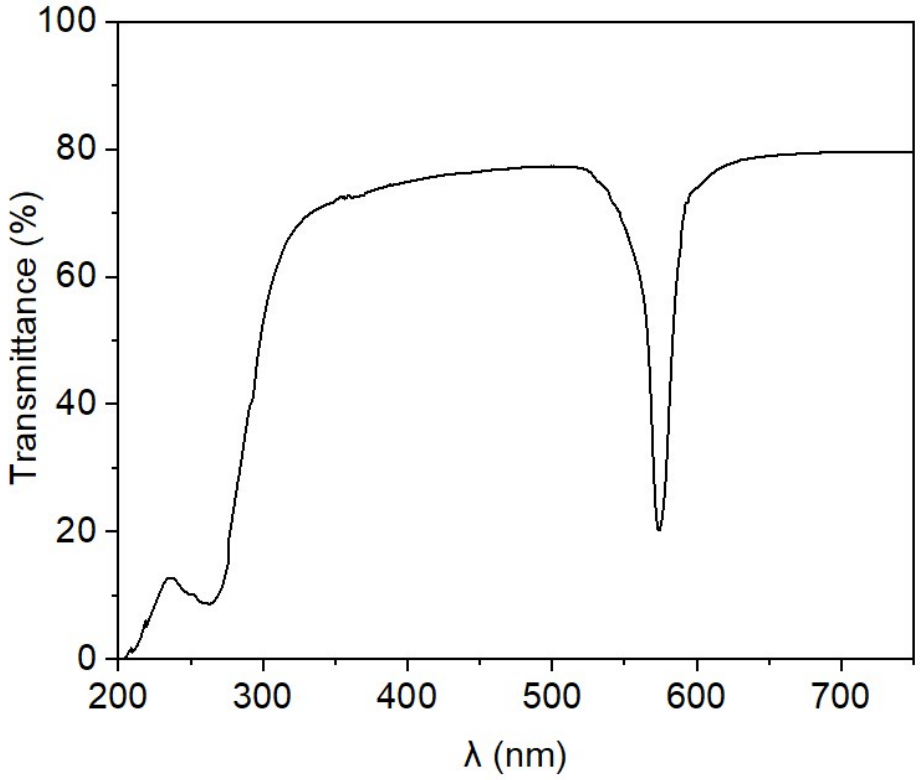

Figure S6. UV-Visible transmission spectrum of photonic crystal hydrogel.

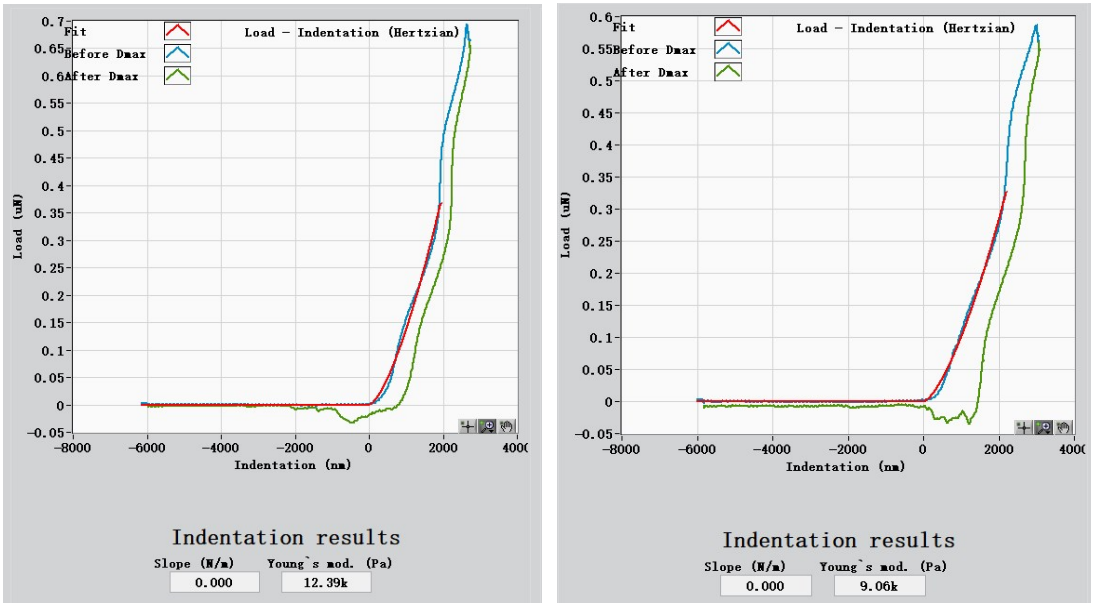

Figure S7. Young's Modulus of Gel by Nano Indenter

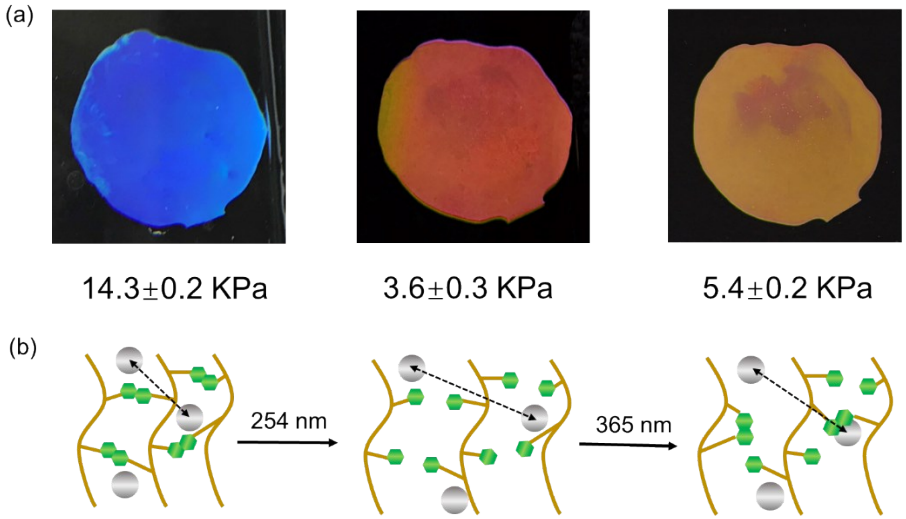

Figure S8. (a) Color and stiffness of the photonic hydrogel after coumarin dimerization, de-dimerization and re-dimerization. (b) Schematic illustration of the changes in color of coumarin-containing hydrogels at different stages: dimerization, de-dimerization and re-dimerization.

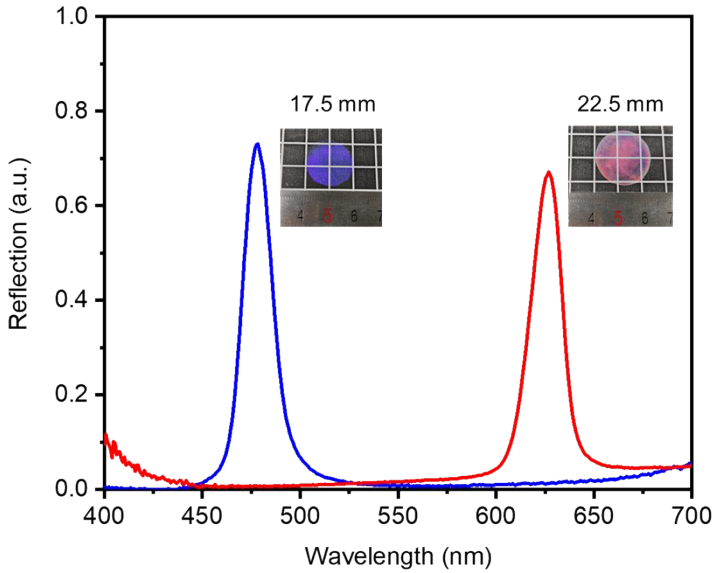

Figure S9. Changes of reflection peak and diameter of photonic crystal hydrogel before and after de-polymerization. The swelling rate of the hydrogel we obtained was 112.6%. The swelling degree of the hydrogel can also be estimated according to the photonic band gap principle of submicron ordered structure ($\lambda_{max} = 1.633Dn_{avg}$), where λmax is the maximum absorption peak wavelength, D is the center-to-center distance between nearest particles (which increases along with hydrogel swelling), navg is the average refractive index of the system composed of colloids and surroundings. The calculated swelling rate (blue → red) of the hydrogel is 119.7%.