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Supporting Information

Shape-Memory and Self-Healing Functions of DNA-Based Carboxymethyl Cellulose Hydrogels Driven by Chemical or Light Triggers

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Experimental Section

Chemicals

4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) sodium salt, 2-(N-Morpholino)ethanesulfonic acid (MES) sodium salt, potasium chloride, magnesium chloride, 6-monodeoxy-6-monoamino-β-cyclodextrin hydrochloride (β-CD), crown ether (CE), 4-(phenyldiazenyl)benzoic acid, N-Boc-1,2-diamioethane, carboxymethylcellulose (CMC), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), N-hydroxysulfosuccinimide (NHS), N-hydroxysulfosuccinimide (sulfo-NHS), trifluoracetic acid (TFA), dimethylformamide (DMF), dichloroform (DCM) and methanol (MeOH) were purchased from Sigma-Aldrich. Gel Red was purchased from Biotium, Inc., USA. Gel Red was purchased from Biotium, Inc., USA. 5' end aminno modified nucleic acid strands were purchased from Integrated DNA Technologies Inc. (Coralville, IA). Tetramethylrhodamine-dextran (70 kDa MW), Amplex Red, resorufin and hemin were purchased from Life Technologies Corporation. Ultrapure water purified by a NANOpure Diamond instrument (Barnstead International, Dubuque, IA, USA).

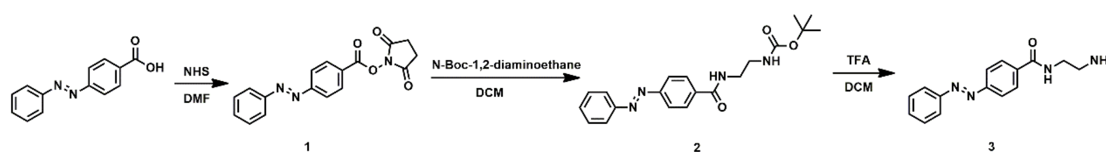
Table S1. Sequences of the DNA strands

(1)	5'-H_2N-$(CH_2)_6$-AAATTCGCGCGCGAA-3'	Mw= 4620.5 Da
(2)	5'-H_2N-$(CH_2)_6$-AAGGGTTAGGG-3'	Mw= 3640.5 Da

Measurement

The absorbance were recorded by a UV-2450 spectrophotometer (Shimadzu). ^{1}H NMR and DOSY spectra were performed by using a Bruker Ultrashield Plus 400 MHz spectrometer. SEM images were taken by using Extra High-Resolution Scanning Electron Microscope Magellan (TM) 400L, microscope. The hydrogel sample was frozen by immersing in liquid nitrogen. The frozen sample was dried through lyophilization and then placed on the slides (Si) and further coated by Au. The mechanical properties and cross-linking kinetics was analyzed by a HAAKE MARS III rheometer (Thermo Scientific).

Synthesis



Scheme S1. The synthesis of (N-aminoethyl p-carboxamide)-trans-azobenzene

*The synthesis of compound (**1**)*

Azobenzoic acid (213 mg, 1 mmol) was dissolved in DMF at 60 °C. NHS (230 mg, 2 mmol), EDC HCl (382 mg, 2 mmol) and a catalytic amount of DMAP were added to the solution. The reaction was stirred overnight. Pure water was added to the solution and red precipitate was obtained and filtered. The solid was dried to yield product **1** (284 mg, 0.88 mmol, 88%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.92 (s, 4H), 7.65 (t, 3H), 8.0 (m, 2H), 8.10 (d, 2H), 8.31 (d, 2H).

*The synthesis of compound (**2**)*

1 (64.6 mg, 0.2 mmol) was dissolved in dried DCM, and N-Boc-1,2-diamioethane (63.2 μL, 0.4 mmol) was added into the solution to stir for 2 h at room temperature. After 2 h, the solvent was evaporated and the crude product was dissolved in DCM and was chromatographed (silica gel, DCM: MeOH = 10:1) to yield **2** (0.042 g, 43%). ^{1}H NMR: ($CDCl_3$, 400 MHz): δ 1.44 (s, 9H), 3.45 (s, 2H), 3.60 (s, 2H), 7.54 (m, 3H), 7.98 (m, 6H).

*The synthesis of compound (**3**)*

2 (41.4 mg, 0.11 mmol) was dissolved DCM (3 mL) and TFA (0.5 mL) was added dropwise at 0 °C into the solution. The mixture was stirred at room temperature for 2 h. After 2 h, the solvent was evaporated to yield the purified product **3** (26.8 mg, 0.1 mmol, 90%). ^{1}H NMR: ($CDCl_3$, 400 MHz): δ 3.44 (t, 2H), 3.53 (t, 2H), 4.34 (s, 2H), 7.62 (m, 1H), 7.79 (t, 2H), 7.94 (d, 2H), 8.0 (d, 2H), 8.08 (d, 2H), 8.78 (s, 1H). ESI-MS for $C_{15}H_{16}N_4O$ (calculated at 268.13): m/z 269.14 ($M+H^+$).

Synthesis of β-CD *functionalized CMC ploymer*

CMC (10 mg, 4×10^{-8} mol) was dissolved in 1 ml MES buffer solution (10 mM, pH = 5.5). EDC (10 mg, 5×10^{-5} mol) was added into the solution and incubated for 5 min, then sulfo-NHS (13 mg, 6×10^{-5} mol) was added and incubated for 10 min. Then β-CD

(8.5 mg, 7×10^{-6} mol) dissolved in HEPES buffer (10 mM, pH = 7.0) was added and the mixture was stirred for 4 h. The modified polymer was separated using a MWCO 100K spin filter (Amicon) for three times. The separated polymer was lyophilized overnight to yield the white powder.

Synthesis of azobenzene functionalized CMC ploymer

CMC (10 mg, 4×10^{-8} mol) was dissolved in 1 ml MES buffer solution (10 mM, pH = 5.5). EDC (10 mg, 5×10^{-5} mol) was added into the solution and incubated for 5 min, then sulfo-NHS (13 mg, 6×10^{-5} mol) was added and incubated for 10 min. Then compound **3** (2.5 mg, 8×10^{-6} mol) was dissolved in DMF (40 μL) and added to the solution and the mixture was stirred for 4 h. The resulted mixture was lyophilized overnight and the modified polymer was separated using a MWCO 100K spin filter (Amicon). The separated polymer was lyophilized overnight to yield the yellow powder.

*Functionalization of the CMC polymer with the DNA duplex tethers (**1**)*

CMC (10 mg, 4×10^{-8} mol) was dissolved in 1 ml MES buffer solution (pH = 5.5). EDC (10 mg, 5×10^{-5} mol) was added into the solution and incubated for 5 min, then sulfo-NHS (13 mg, 6×10^{-5} mol) was added and incubated for 10 min. Then HEPES buffer solution (50 mM, pH = 7.0) that included the DNA duplex tethers (**1**) (3×10^{-7} mol) was added into the solution to react for 4 h. The modified polymer was separated using a MWCO 100K spin filter (Amicon). The separated polymer was lyophilized overnight to yield the functionalized white powder.

*Functionalization of the CMC polymer with the half G-quadruplex tethers (**2**)*

CMC (10 mg, 4×10^{-8} mol) was dissolved in 1 ml MES buffer solution (pH = 5.5). EDC (10 mg, 5×10^{-5} mol) and sulfo-NHS (13 mg, 6×10^{-5} mol) were added into the solution and incubated for 5 min. Then HEPES buffer solution (50 mM, pH = 7.0) that included the half G-quadruplex units (**2**) (1.2×10^{-6} mol) was added into the solution to react for 4 h. The modified polymer was separated using a MWCO 100K spin filter (Amicon). The separated polymer was lyophilized overnight to yield the functionalized white powder.

*Preparation of the hydrogel crosslinked by self-complementary nucleic acid (**1**) and* β-CD/azobenzene *bridges and the switchable transitions between different states*

The DNA (**1**)/ β-CD functionalized polymer (2 mg) and DNA (**1**)/ azobenzene functionalized polymer (2 mg) were dissolved in in a HEPES buffer solution (10 mM, $MgCl_2$, 100 mM, pH = 7.0) to yield a polymer solution containing 1 mM of (**1**). The solution was heated to 90˚ and then transferred into the triangle-shaped mold. After keeping it overnight to form a hydrogel, the hydrogel was extruded from the mold and treated with UV light and visible light for 10 min and 10 min, respectively, to trigger the switchable transitions between low stiffness and high stiffness states.

*Preparation of the hydrogel crosslinked by G-quadruplex (**2**) and* β-CD/azobenzene *bridges and the switchable transitions between three states*

The DNA (**2**)/ β-CD functionalized polymer (2 mg) and DNA (**2**)/ azobenzene functionalized polymer (2 mg) were dissolved in in a HEPES buffer solution (10 mM, $MgCl_2$, 100 mM, pH = 7.0) that contains KCl (0.2 M) to yield a polymer solution with 1.32 mM of (**2**). The solution was heated to 90˚ and then transferred into the triangle-shaped mold. After keeping it overnight to form a hydrogel, the hydrogel was extruded from the mold and treated with UV light and visible light for 10 min and 10 min, respectively, to trigger the switchable transitions between low stiffness and high stiffness states. Similarly, the hydrogel was treated with CE (0.2 M, 100 μl) and K^+ (0.2 M, 100 μl) for 4 h to trigger the switchable transitions between low stiffness and high stiffness states.

Calculation of hydrogel area

Using Image-Pro Plus software, we can select the colored region of hydrogels or quasi-liquid states, measure the pixels of the selected regions and export measurements to statistical and spreadsheet package.

*Preparation of TMR-dextran loaded hydrogel crosslinked by G-quadruplex (**2**) and β-CD/azobenzene bridges and the release of TMR-dextran*

The DNA (**2**)/ β-CD functionalized polymer (2 mg) and DNA (**2**)/ azobenzene functionalized polymer (2 mg) were dissolved in in a HEPES buffer solution (10 mM, $MgCl_2$, 100 mM, pH = 7.0) that contains KCl (0.2 M) and TMR-dextran (0.2 mg/mL of) to yield a polymer solution with 1.32 mM of (**2**). The solution was heated to 90˚ and then kept overnight to form a hydrogel. The hydrogel was treated with UV light for 10 min to trigger the release of TMR-dextran. Similarly, the hydrogel was treated with CE (0.2 M, 100 μl) to trigger the release of TMR-dextran.

*Binding/unbinding of G-quadruplex in the hydrogel crosslinked by G-quadruplex (**2**) and β-CD/azobenzene bridges*

The DNA (**2**)/ β-CD functionalized polymer (2 mg) and DNA (**2**)/ azobenzene functionalized polymer (2 mg) were dissolved in in a HEPES buffer solution (10 mM, $MgCl_2$, 100 mM, pH = 7.0) that contains KCl (0.2 M) and hemin (50 μM). The solution was heated to 90° and then kept overnight to form a hydrogel. Immersion of the hydrogel in the HEPES buffer that contains Amplex Red (5 μM), and then measure the fluorescence of the buffer. In addition, after treatment of the hydrogel with 18-crown-6-ether, immerse the hydrogel in the HEPES buffer that contains Amplex Red (5 μM) and measure the fluorescence change.

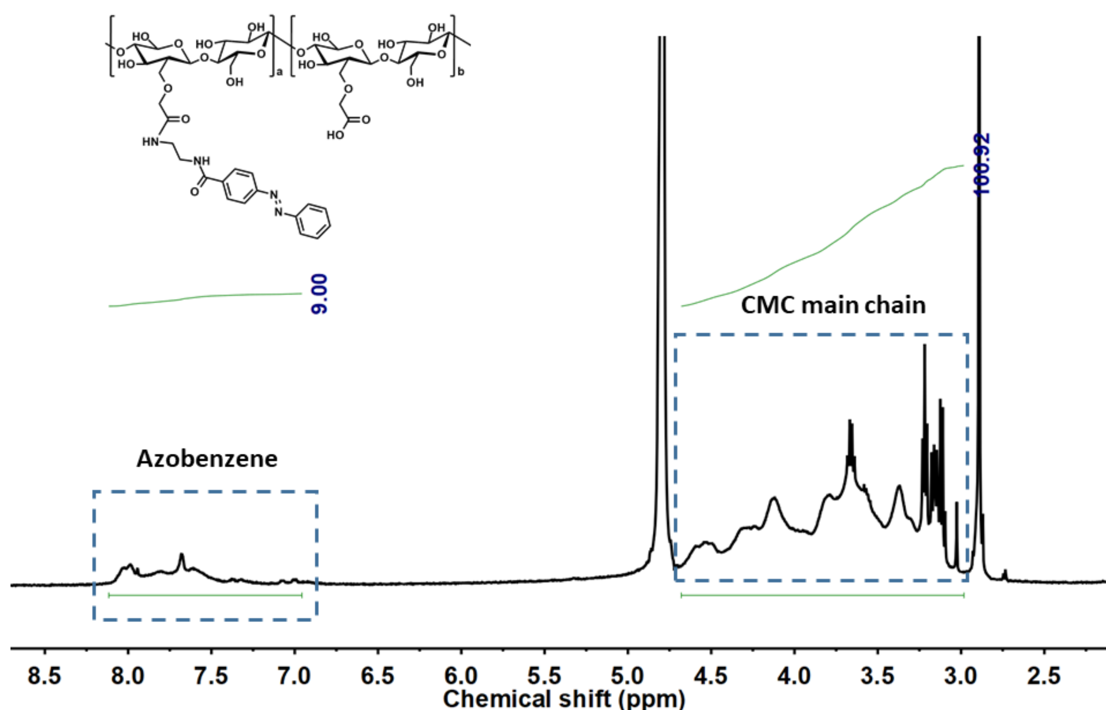


Figure S1. ^{1}H NMR of azobenzene-functionalized carboxymethyl cellulose (CMC) polymer. From the integration of values of the azobenzene units and the proton associated with the glucose unit of CMC, the loading of azobenzene units on CMC was estimated to be 1:10.

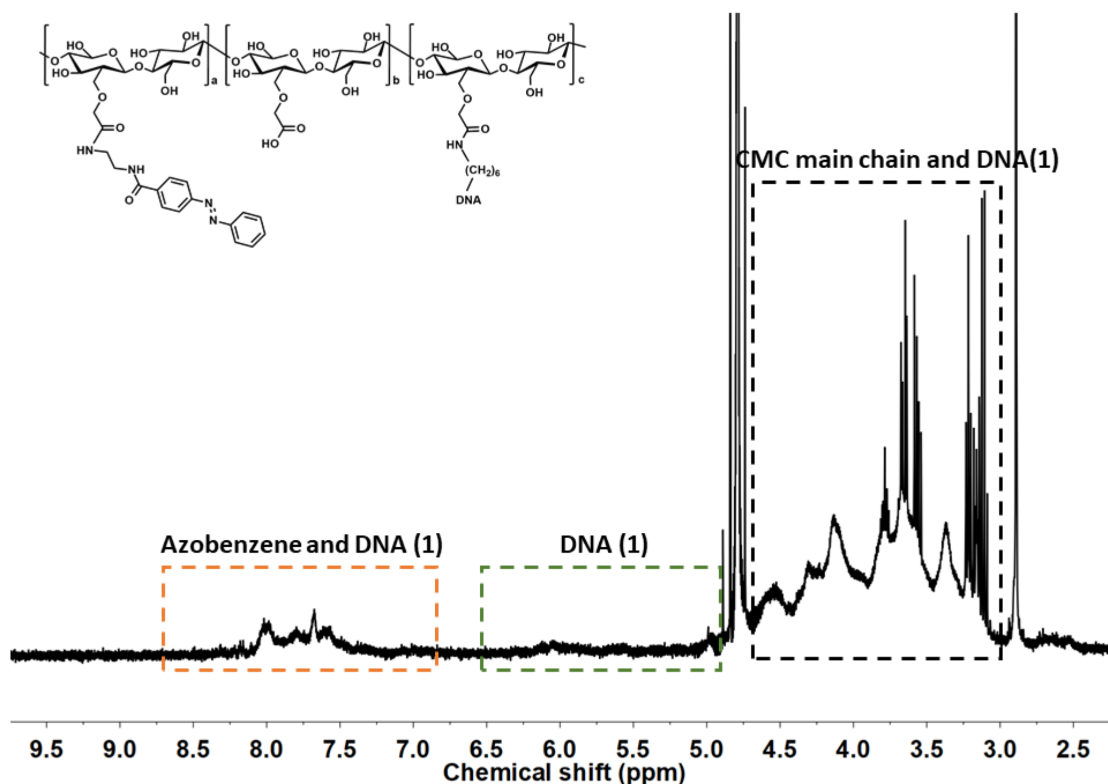


Figure S2. ^{1}H NMR of polymer P_A.

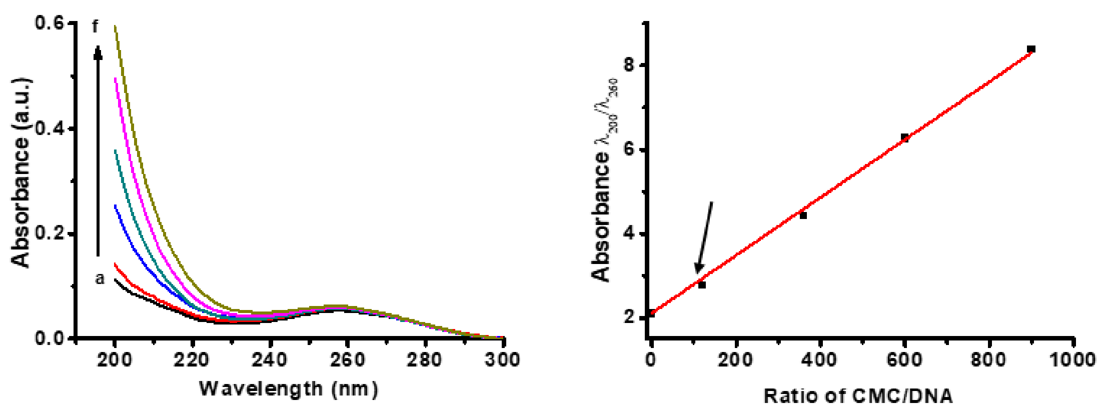


Figure S3. Determination of the loading of CMC polymer chains by the nucleic acids tethers (**1**): A) Absorbance spectra of different concentrations of carboxymethylcellulose (M_W 250000) in the prescence of a constant concentration of the nucleic acid (**1**), corresponding to 0.5 μM: (a) 0 μM (b) 0.1 μM (c) 0.3 μM (d) 0.5 μM (e) 0.75 μM (f) 1 μM. B) Calibration curve corresponding to absorbance ratio λ_{200} nm/λ_{260} nm as a function of the molar ratio carboxymethylcellulose/DNA. Arrow marked indicates the loading of the nucleic acid (**1**) on the CMC polymer chains that corresponds to 1:110.

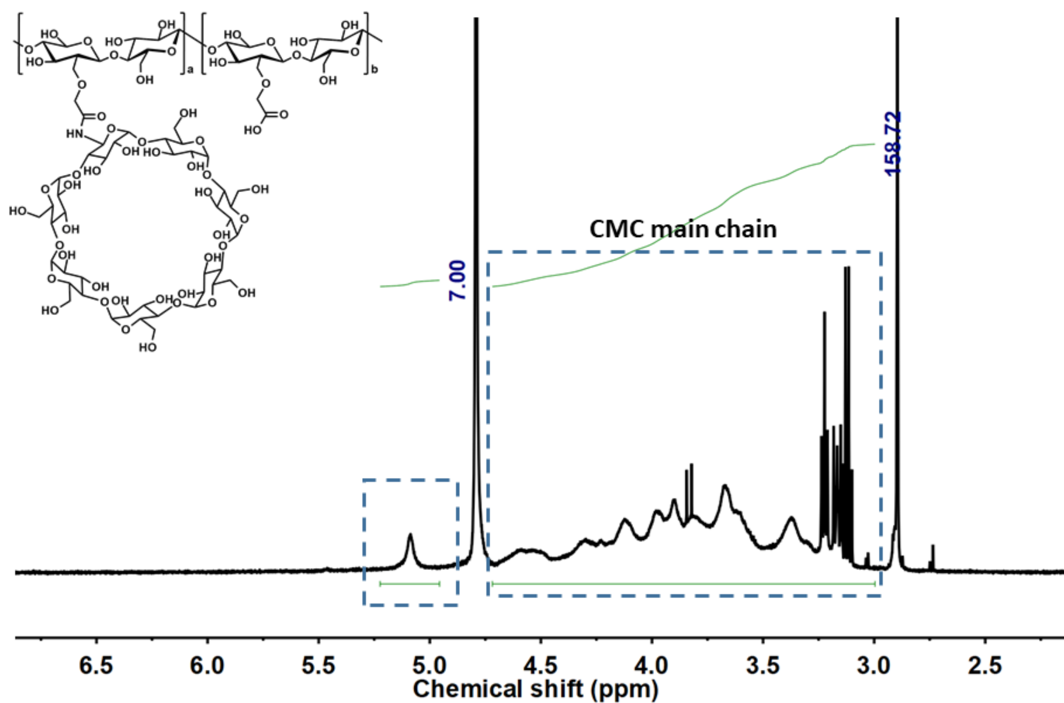


Figure S4. ^{1}H NMR of β-CD-functionalized carboxymethyl cellulose (CMC) polymer. From the integration of values of the Hα of β-CD and the proton associated with the glucose unit of CMC, the loading of β-CD on CMC was estimated to be 1:10.

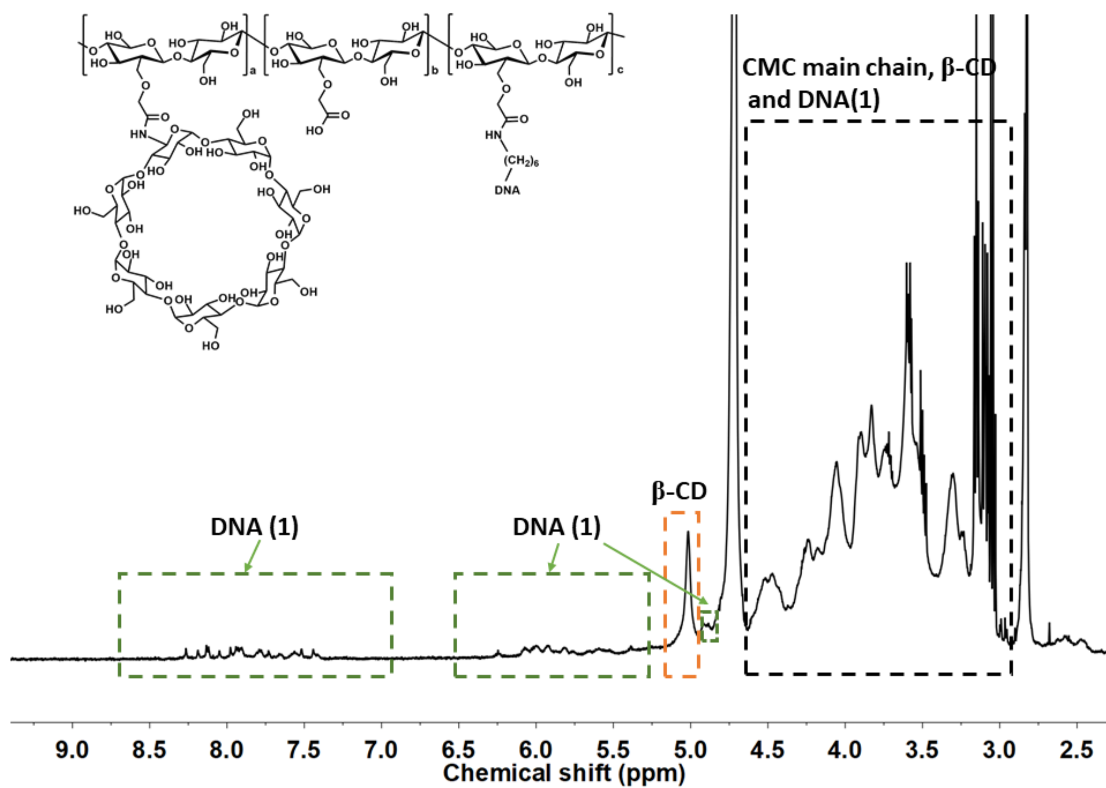


Figure S5. ^{1}H NMR of polymer P_B.

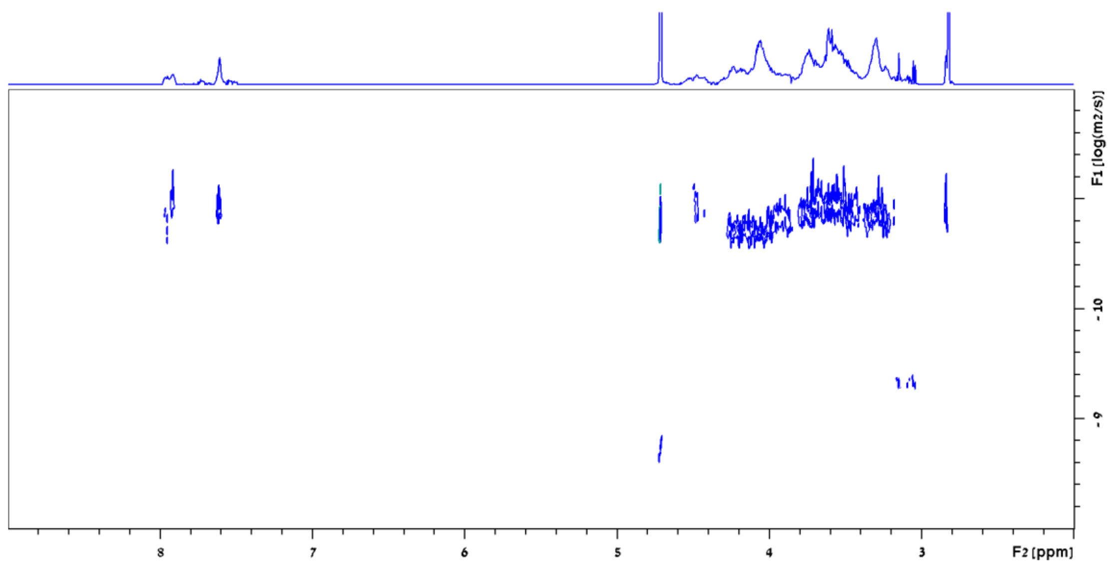


Figure S6. DOSY NMR of polymer P_A.

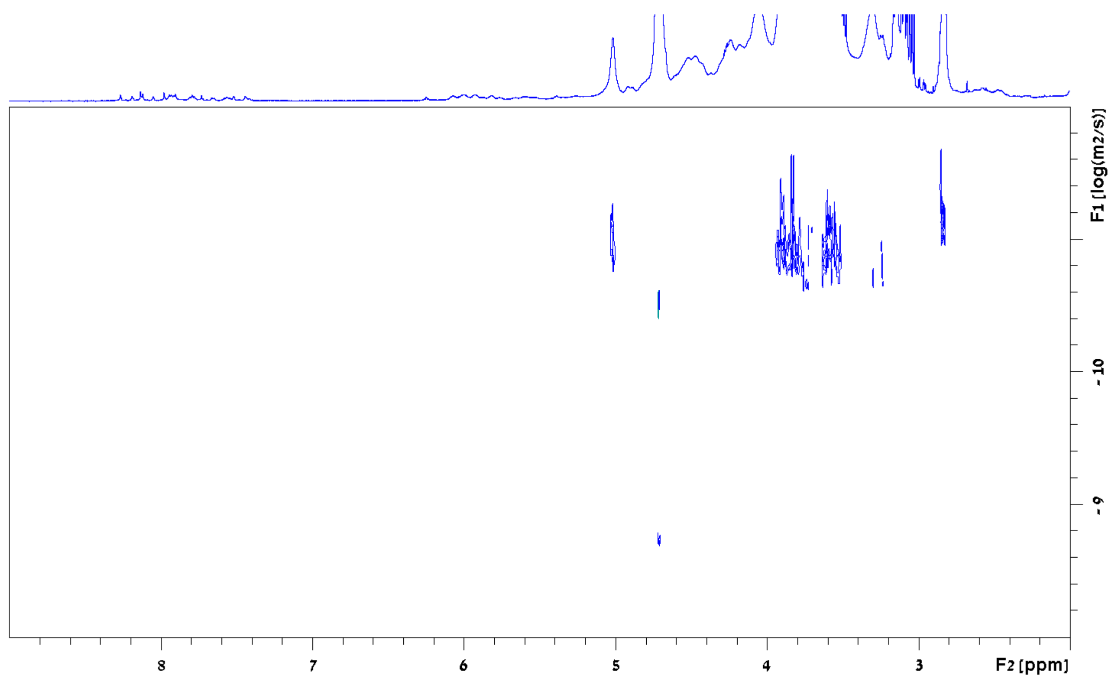


Figure S7. DOSY NMR of polymer P_B.

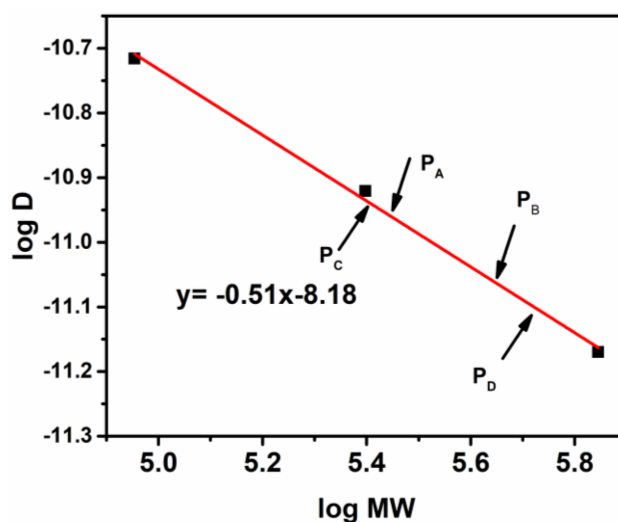


Figure S8. Calibration curve corresponding to the diffusion coefficients of a series of CMC polymers of known average molecular weights (Mw= 90K, 250K and 700K).

Table S2. The diffusion coefficients and average molecular weights of different polymers

Polymer	logD	MW
P_A	-10.96	275422 Da
P_B	-11.07	464159 Da
P_C	-10.93	251188 Da
P_D	-11.10	524807 Da

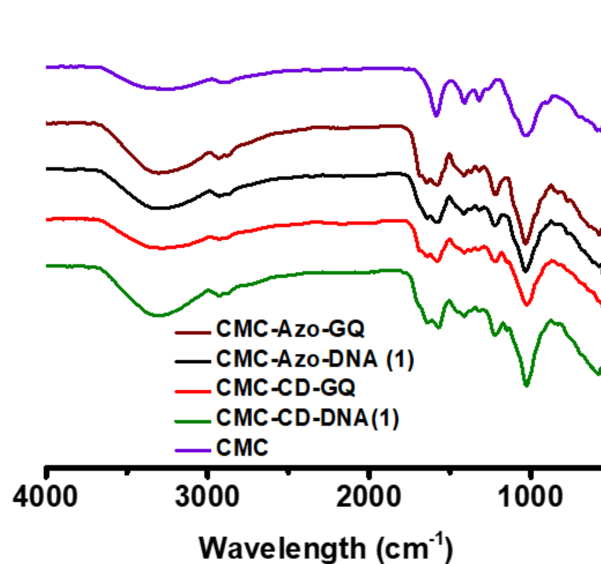


Figure S9. FTIR spectra of different functionalized carboxymethyl cellulose (CMC) polymer.

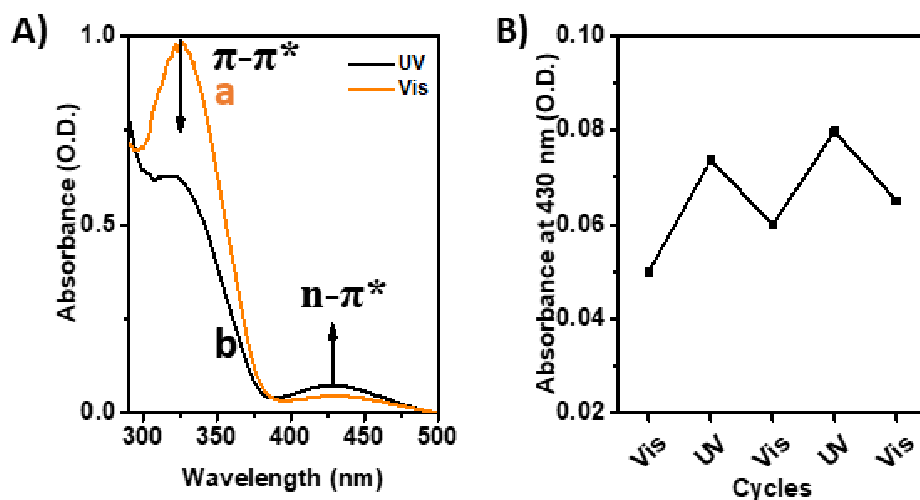


Figure S10. (A) The absorbance spectra of the trans-azobenzene//β-CD and (**1**)/(**1**) crosslinked hydrogel (curve (a)), and of the (**1**)/(**1**) crosslinked and free β-CD and cis-azobenzene modified hydrogel (curve (b)); (B) The switchable transitions of the hydrogel (10 min illumination).

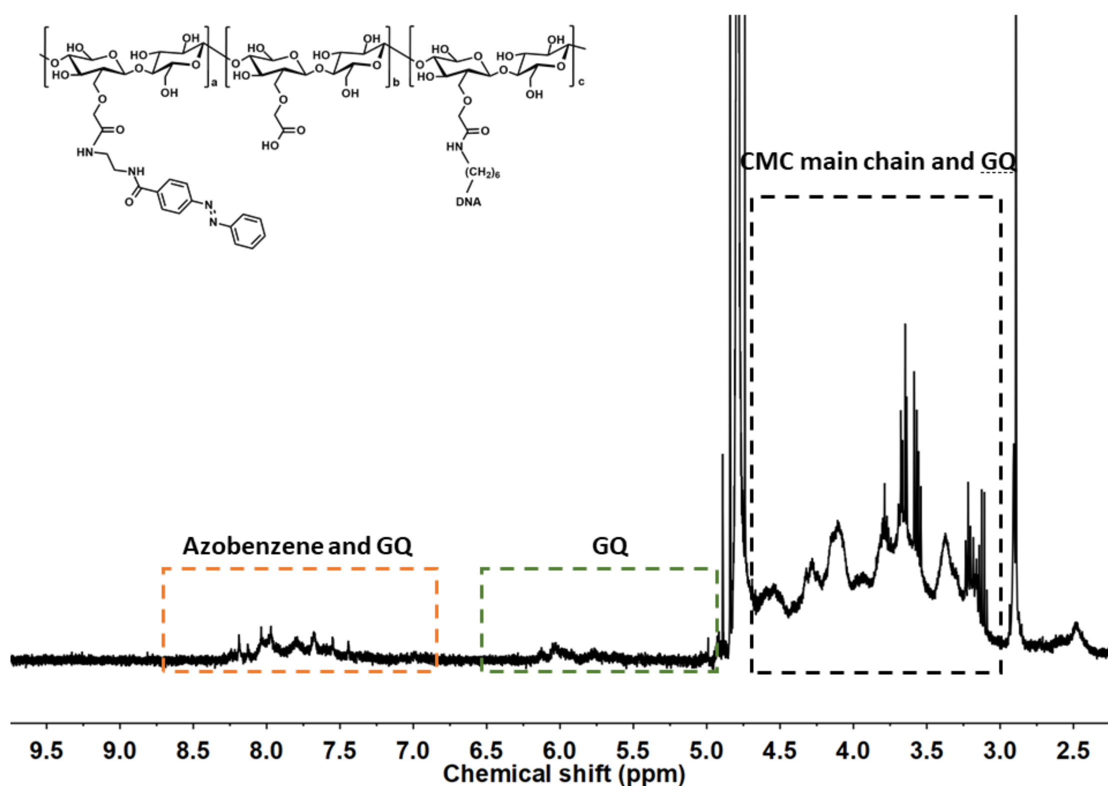


Figure S11. ^{1}H NMR of polymer P_C.

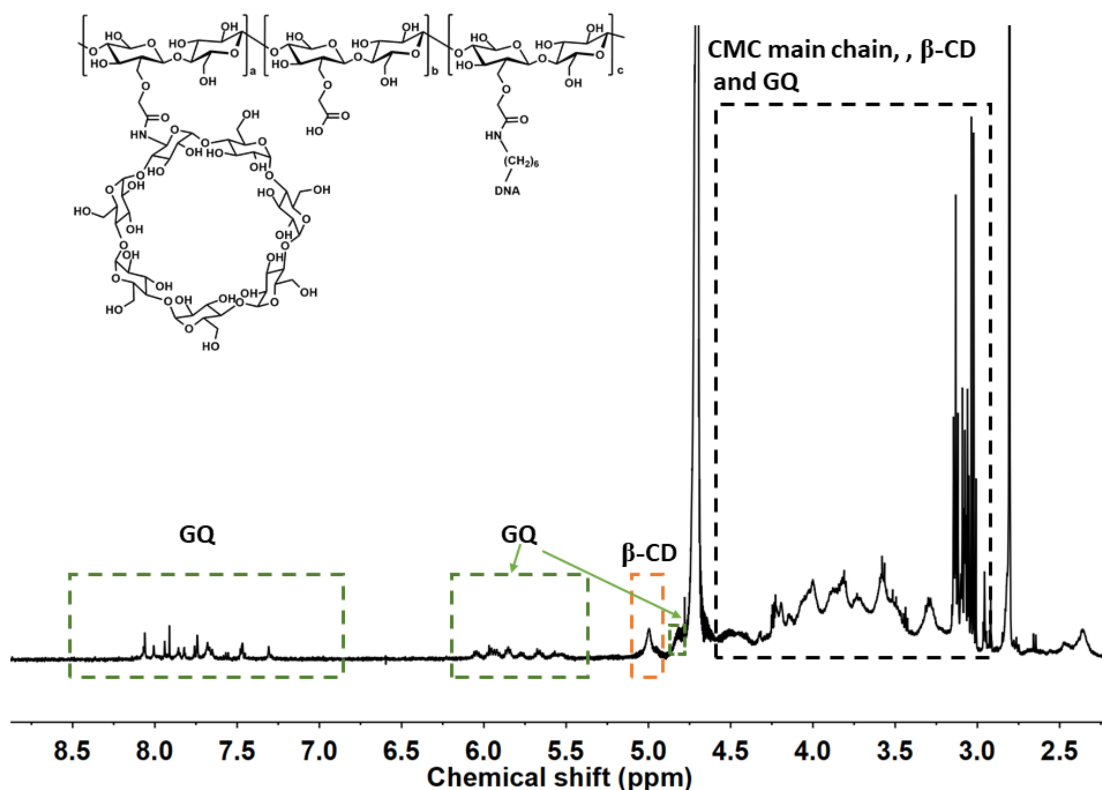


Figure S12. ^{1}H NMR of polymer P_D.

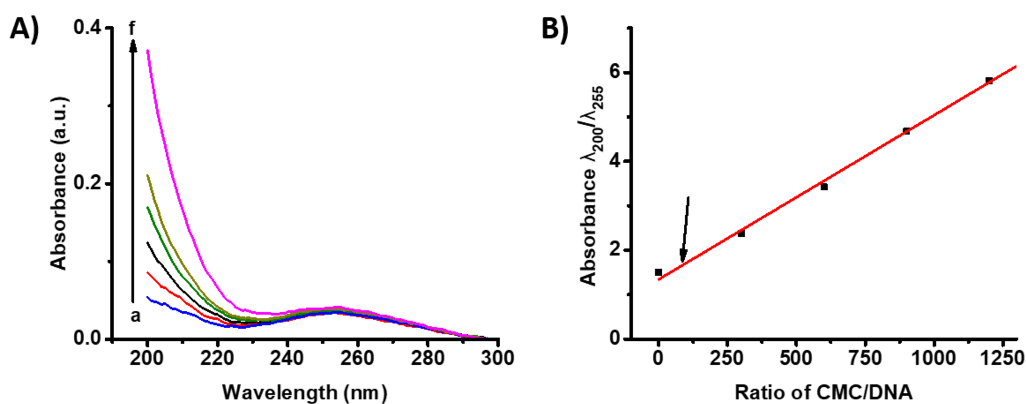


Figure S13. Determination of the loading of CMC polymer chains by the G quadruplex (**2**): A) Absorbance spectra of different concentrations of carboxymethylcellulose (M_W 250000) in the prescence of a constant concentration of the G quadruplex (**2**), corresponding to 0.5 μM: (a) 0 μM (b) 0.1 μM (c) 0.3 μM (d) 0.5 μM (e) 0.75 μM (f) 1 μM. B) Calibration curve corresponding to absorbance ratio λ_{200} nm/λ_{260} nm as a function of the molar ratio carboxymethylcellulose/DNA. Arrow marked indicates the loading of the G quadruplex (**2**) on the CMC polymer chains that corresponds to 1:87.

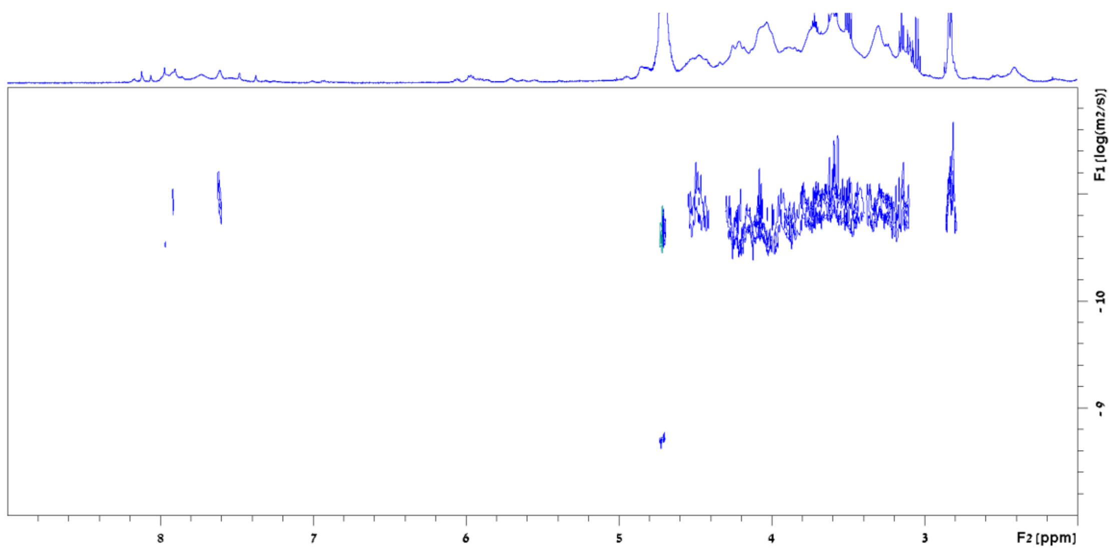


Figure S14. DOSY NMR of polymer P_C.

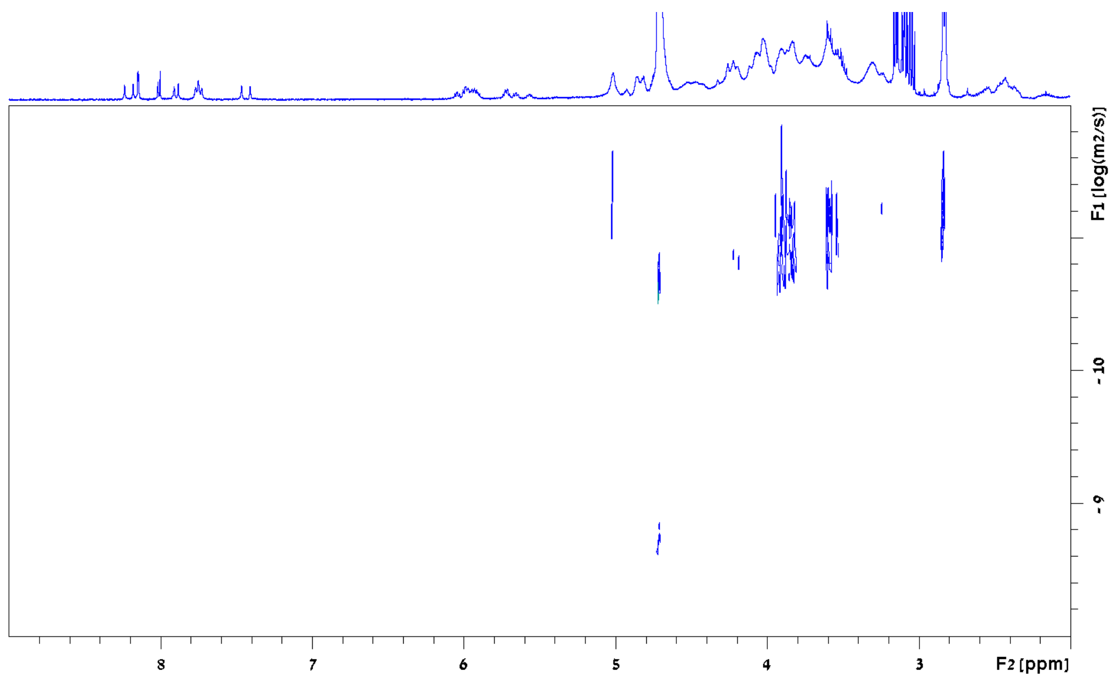


Figure S15. DOSY NMR of polymer P_D.

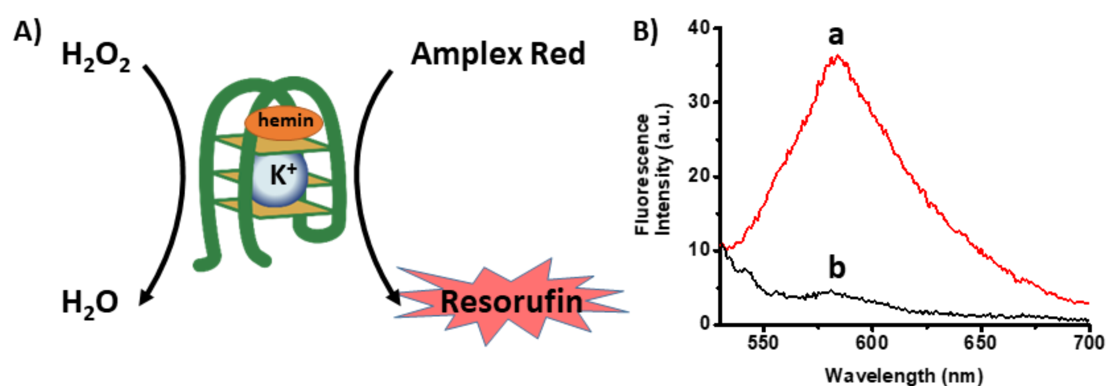


Figure S16. (A) Schematic illustration of Hemin/G-quadruplex exhibiting horseradish peroxidase DNAzyme activations where the catalyzed oxidation of Amplex Red by H_2O_2 to form the fluorescent Resorufin product; (B) The fluorescence induced by Hemin/G-quadruplex catalyzed oxidation of the Amplex Red to resorufin in the presence of H_2O_2, curve (a) and the fluorescence after treatment of the hydrogel with 18-crown-6-ether, leading to the loss catalytic activity, curve (b).

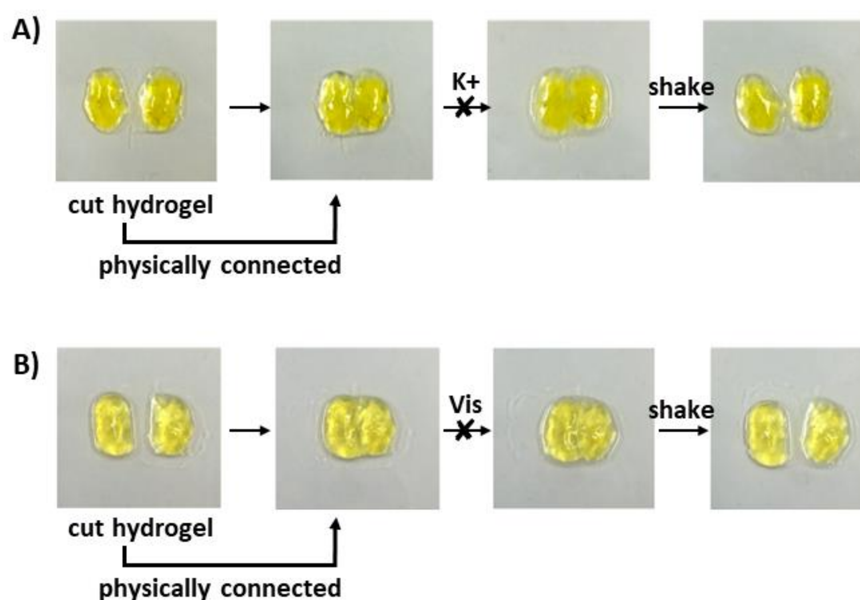


Figure S17. A) The control experiment of treating the two physically-inter-connected hydrogel pieces without K^+ ions; B) The control experiment of treating the physically inter-connected two pieces without irradiation

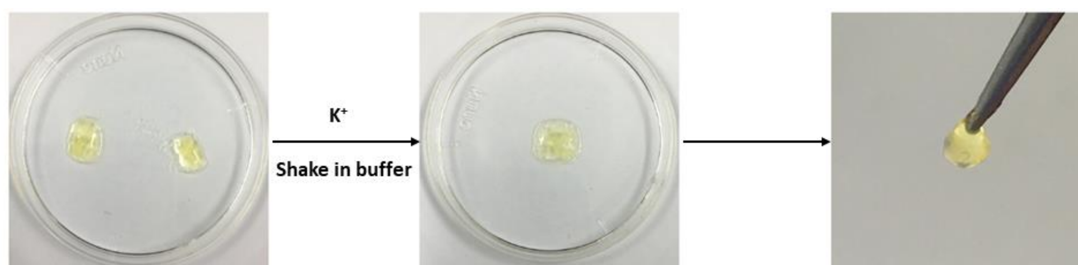


Figure S18. Self-healing of two separated pieces of low-stifness hydrogel crosslinked by the trans-azobenzene/β-CD linker upon shaking and addition of K^+-ions as the healing trigger.