Supporting Information

Chemical and Photochemical DNA "Gears" Reversibly Control Stiffness, Shape-Memory, Self-Healing and Controlled Release Properties of Polyacrylamide Hydrogels

Xia Liu, ‡,[a] Junji Zhang, ‡,[b] Michael Fadeev,[a] Ziyuan Li,[b] Verena Wulf,[a] He Tian,[b] Itamar Willner,,[a]*

[a]*Institute of Chemistry, The Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Jerusalem, 91904, Israel.*

[b]*Key Laboratory for Advanced Materials and Joint International Research Laboratory of Precision Chemistry and Molecular Engineering, Feringa Nobel Prize Scientist Joint Research Center, School of Chemistry and Molecular Engineering, East China University of Science & Technology, 130 Meilong Road, Shanghai, 200237, China.*

‡ *These authors contributed equally to this work.*

E-mail: willnea@vms.huji.ac.il

Experimental Section

Materials

MES sodium salt, HEPES sodium salt, potasium chloride (KCl), magnesium chloride ($MgCl_2$), ammonium persulfate (APS), N,N,N′,N′-tetramethylethylenediamine (TEMED), acrylamide solution (40%), acrylic acid, 3-(acrylamido)phenylboronic acid (AAPBA), D-(+)-glucosamine hydrochloride, 18-crown-6-ether, N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and n-hydroxysulfosuccinimide (NHS) were purchased from sigma-aldrich. SYBR Green I was purchased from Life Technologies Corporation (USA). Desalted 5′-end acrydite-modified nucleic acid strands were purchased from Integrated DNA Technologies Inc. (Coralville, IA). All the solutions were prepared with ultrapure water purified by a NANOpure Diamond instrument (Barnstead International, Dubuque, IA, USA).

The sequences used in the study are:

(**1**) 5'-acrydite-AAG GGT TAG GG-3'

(**2**) 5'-acrydite-TT GGA CCG AT-3'

(**3**) 5'-acrydite-AT GTT AGA GC-3'

(**4**) 5'-GGT AAC AAA ATT TTT ATC GGT CCA A-3'

t-azo (**5**) 5'-GAT-azo-TGT-azo-TAC-azo-CAGTTTGCTCTAACAT -3'

Measurement

The absorbance was recorded using a UV-2450 spectrophotometer (Shimadzu). 1H NMR and DOSY spectra were performed by a Bruker Ultrashield Plus 500 MHz

spectrometer. SEM images were taken by using Extra High-Resolution Scanning Electron Microscope Magellan (TM) 400L, microscope. The hydrogel samples were frozen by immersing in liquid nitrogen. The frozen samples were dried through lyophilization and then placed on the slides (Si) and further coated by Au. The mechanical properties and crosslinking kinetics were analyzed by a HAAKE MARS III rheometer (Thermo Scientific). Young's moduli of all samples were measured on the Piuma Nanoindenter (Optics11, Amsterdam, NL).

Synthesis of the acrylamide/acrydite-nucleic acid/3-(acrylamido)phenylboronic acid copolymer

For the polymer A, a buffer solution (HEPES, 10 mM, $MgCl_2$, 100 mM, pH = 7.0), 200 μL, that included 2% acrylamide, 1.3 mM acrydite-modified DNA strands (**1**), and 10 mM AAPBA was prepared. For the polymer C, a buffer solution (HEPES, 10 mM, $MgCl_2$, 100 mM, pH = 7.0), 200 μL, that included 2% acrylamide, 1 mM acrydite-modified DNA strand (**2**) and 10 mM AAPBA was prepared. The solutions were bubbled with nitrogen for 15 minutes. Subsequently, 15 μL of a 0.5-mL aqueous solution that included 50 mg APS and 25 μL TEMED was added to each of the monomer solutions. The solutions were polymerized at room temperature for 5 minutes, before the resulting solutions were further polymerized at 4 ℃ for 12 hours. After polymerization, the unreacted monomer units, the initiator and salts were washed away using a Microcon (Millipore) spin filter unit (MWCO: 10K). The separated polymers were freeze-dried overnight to get purified polymers.

Synthesis of the acrylamide/acrydite-nucleic acid/D-(+)-glucosamine copolymer

For the polymer B, a buffer solution (HEPES, 10 mM, $MgCl_2$, 100 mM, pH = 7.0), 200 μL, that included 2% acrylamide, 1.3 mM acrydite-modified DNA strands (**1**) and 65 mM acrylic acid was prepared. For the polymer D, a buffer solution (HEPES, 10 mM, $MgCl_2$, 100 mM, pH = 7.0), 200 μL, that included 2% acrylamide, 1.0 mM acrydite-modified DNA strand (**3**) and 65 mM acrylic acid was prepared. The solutions were bubbled with nitrogen for 15 minutes. Subsequently, 15 μL of a 0.5-mL aqueous solution that included 50 mg APS and 25 μL TEMED was added to each of the monomer solutions. The solutions were polymerized at room temperature for 5 minutes, before the resulting solutions were further polymerized at 4 ℃ for 12 hours. After polymerization, the unreacted monomer units, the initiator and salts were washed away using a MWCO 10K spin filter (Amicon). The purified polymers were separated from the filter and used for modification of glucosamine. To an aqueous MES buffer solution (10 mM, pH = 5.5), 1.3 mL, that included polymer B or D, and N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride, 25 mg, and sulfo-N-hydroxysuccinimide, 28 mg, and after incubation for 15 minutes was added 1.3 mL of a HEPES buffer solution (50 mM, pH = 7.2) that included 0.05 M glucosamine. The mixture was allowed to react for a time-interval of 6 hours. The modified polymers were purified using a MWCO 10K spin filter (Amicon).

Preparation of a triangle-shaped hydrogel and K^+/18-crown-6-ether triggered transitions between hydrogel and quasi-liquid states

To form a triangle-shaped hydrogel crosslinked by K^+-stabilized G-quadruplexes and the glucosamine/boronate ester, the dried copolymer samples were dissolved in a 100

μL HEPES buffer (10 mM HEPES, 100 mM $MgCl_2$, pH = 8.0) that contains 200 mM KCl to yield a copolymer solution containing 2.9 mM (**1**). And then 2 μL of SYBR Green I was added for the final staining of the resulting hydrogel. The resulting mixture solution was heated to 90 ℃ and then poured into the triangle-shaped mold. After incubation overnight, the shaped hydrogel crosslinked by K^+-stabilized G-quadruplexes and the glucosamine/boronate ester was extruded from the mold. The hydrogel was treated with CE solution (200 mM) and K^+-ion solution (200 mM) for 1 h to trigger the switchable transitions between low stiffness and high stiffness states.

Preparation of a triangle-shaped hydrogel and UV/Vis triggered transitions between hydrogel and quasi-liquid states

To form a triangle-shaped hydrogel crosslinked by the *trans*-azobenzene stabilized duplex and the glucosamine/boronate ester bridges, the dried copolymer samples were dissolved in a 100 μL HEPES buffer (10 mM HEPES, 100 mM $MgCl_2$, pH = 8.0) to yield a copolymer solution containing 2.6 mM nucleic acids (**2**) and (**3**), 0.5 mM (**4**), 0.5 mM *t*-azo (**5**). And then 2 μL of SYBR Green I was added for the final staining of the resulting hydrogel. The resulting mixture solution was heated to 90 ℃ and then poured into the triangle-shaped mold. After incubation overnight, the shaped hydrogel crosslinked by the *trans*-azobenzene stabilized duplex and the glucosamine/boronate ester bridges was extruded from the mold. The hydrogel was irradiated under UV light (λ = 365 nm) for and Vis light (λ > 420 nm) for 30 minutes to trigger the switchable transitions between low stiffness and high stiffness states. UV light irradiation was carried out with portable UV lamp (LUYOR-365, 18000 μW/cm^2).

Visible light irradiation was carried out with Xeon lamp (Hamamatsu, 200 W, 8.0 A) with a 420 nm filter.

Preparation of doxorubicin loaded hydrogel crosslinked by the glucosamine/boronate ester and the K^+-stabilized G-quadruplex bridges and the release of doxorubicin

The DNA (**1**)/phenylboronate functionalized polymer (4%) and DNA (**1**)/glucosamine functionalized polymer (4%) were dissolved in a HEPES buffer solution (10 mM, $MgCl_2$, 100 mM, pH = 8.0) that contains KCl (200 mM) and doxorubicin (1 mM) to yield a polymer solution. The mixture solution was heated to 90 ℃ and then kept overnight to form a hydrogel. After washing, the hydrogel was treated with 10 mM CE to trigger the release of doxorubicin.

Preparation of doxorubicin loaded hydrogel crosslinked by the glucosamine-boronate ester and the trans-azobenzene stabilized duplex and the release of doxorubicin

The DNA (**2**)/phenylboronate functionalized polymer (4%) and DNA (**3**)/glucosamine functionalized polymer (4%) were dissolved in in a HEPES buffer solution (10 mM, $MgCl_2$, 100 mM, pH = 8.0) that contains doxorubicin (1 mM) to yield a polymer solution. The mixture solution was heated to 90 ℃ and then kept overnight to form a hydrogel. After washing, the hydrogel was treated with UV light (λ = 365 nm) to trigger the release of doxorubicin.

Regeneration of the doxorubicin drug loaded hydrogel matrices

The unloaded boronate ester-glucosamine/G-quadruplex crosslinked hydrogel and the boronate ester-glucosamine/(2)-(4)/(3)-*t-azo* (5) photoresponsive duplex crosslinked hydrogels provide functional polymers that can yield regenerated

stimuli-responsive doxorubicin-loaded matrices for the release of the drug by command. Towards this goal, the unloaded hydrogel was subjected to an acid environment (pH = 5.0), to separate the boronate ester-glucosamine bridges, and subsequently treated with the crown ether or the UV irradiation to yield the separated hydrogel chains. The resulting mixture was neutralized, heated to 80 ℃ and the doxorubicin drug, 1 mM, was added to the mixture. K^+-ions, 200 mM, were added to the G-quadruplex functionalized polymer mixture, and the photoresponsive polymer mixture was irradiated, $\lambda > 420$ nm for 20 minutes. The resulting polymer mixture was allowed to cool down to room temperature to yield the drug-loaded stiff boronate ester-glucosamine/G-quadruplex responsive hydrogel or the photoresponsive boronate ester-glucosamine/(2)-(4)/(3)-*t-azo* (5) duplexes hydrogel. The treatment of the cooperatively stabilized boronate-ester/K^+-ion G-quadruplex bridged hydrogel with crown ether yielded the low stiffness hydrogel that resulted in the release of the doxorubicin drug. The release profile, Fig. S7(B) is similar to the release profile of the drug in the first cycle of transition of the hydrogel from the stiff state to the lower stiffness state, Fig. S7(A). Similar regeneration of the drug release functions of the photoresponsive hydrogel was observed, Fig. S8. The irradiation of the stiff boronate ester-glucosamine/(2)-(4)/(3)-*t-azo* (5) duplexes crosslinked hydrogel, $\lambda = 365$ nm, resulted in the function of the low-stiffness hydrogel state and the release of the doxorubicin drug, Fig. S8(B). A similar drug release profile to that observed for the first cycle corresponding to photo-induced release of drug, Fig. S8(A) is demonstrated.

These results demonstrated that the hydrogels can be regenerated to yield new drug-loaded matrices.

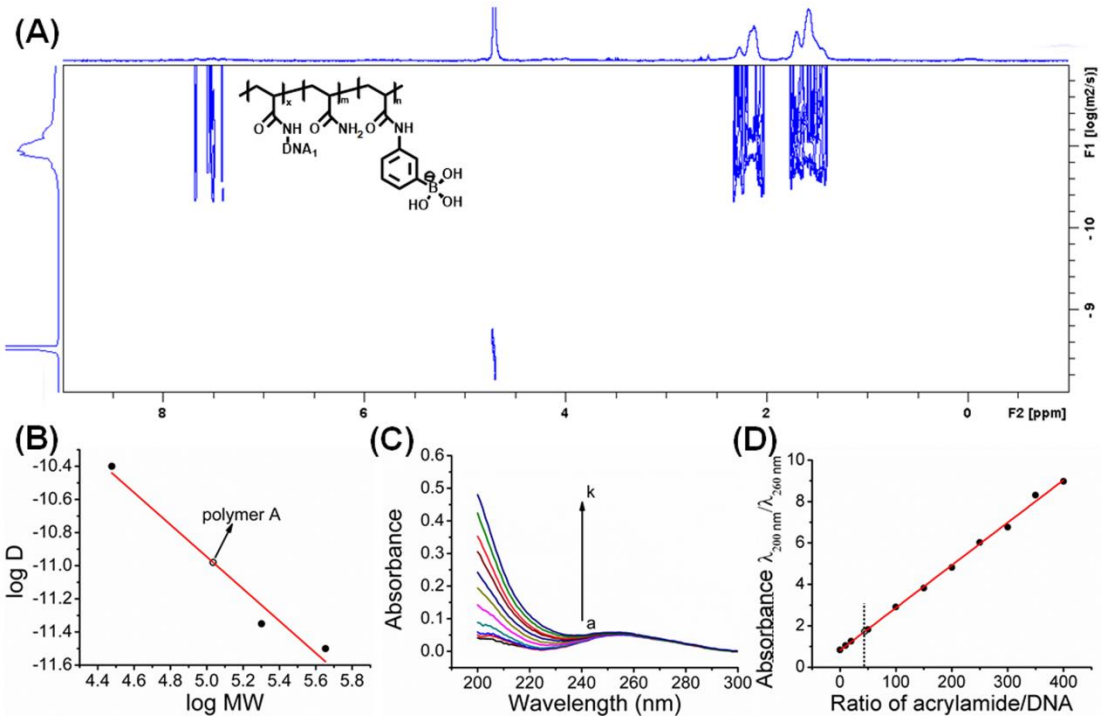

Fig. S1. (A) The DOSY spectrum of phenylboronic acid and guanosine-rich nucleic acid tethers (**1**)-functionalized polyacrylamide chains (P_A). (B) Calibration curve corresponding to the Log/Log display of the diffusion coefficients vs. molecular weights of a series of polyacrylate derivatives. The point marked with an arrow corresponds to the diffusion coefficient of P_A extracted from its DOSY spectrum, resulting in estimated average molecular weight of 110 000 (P_A). (C) Determination of the loading of acrylamide monomer by the nucleic acid tethers (**1**) of P_A: Absorption spectra of different concentrations of polyacrylamide in the presence of a constant concentration of acrydite modified nucleic acid, corresponding to 0.5 $\times 10^{-6}$ M: (a) 0, (b) 0.5 $\times 10^{-5}$ M, (c) 1 $\times 10^{-5}$ M, (d) 2.5 $\times 10^{-5}$ M, (e) 5 $\times 10^{-5}$ M, (f) 7.5 $\times 10^{-5}$ M, (g) 1.0 $\times 10^{-4}$ M, (h) 1.25 $\times 10^{-4}$ M, (i) 1.5 $\times 10^{-4}$ M, (j) 1.75 $\times 10^{-4}$ M, (k) 2.0 $\times 10^{-4}$ M, (l) 2.25 $\times 10^{-4}$ M, (k) 2.5 $\times 10^{-4}$ M. (D) Calibration curve corresponding to the absorbance ratio $A_{\lambda 200nm}/A_{\lambda 260nm}$ as a function of acrylamide/DNA ratio. Dashed

line indicates the loading of the DNA on the acrylamide copolymer, corresponding to acrydite modified nucleic acid tether (**1**):acrylamide = 1:44.

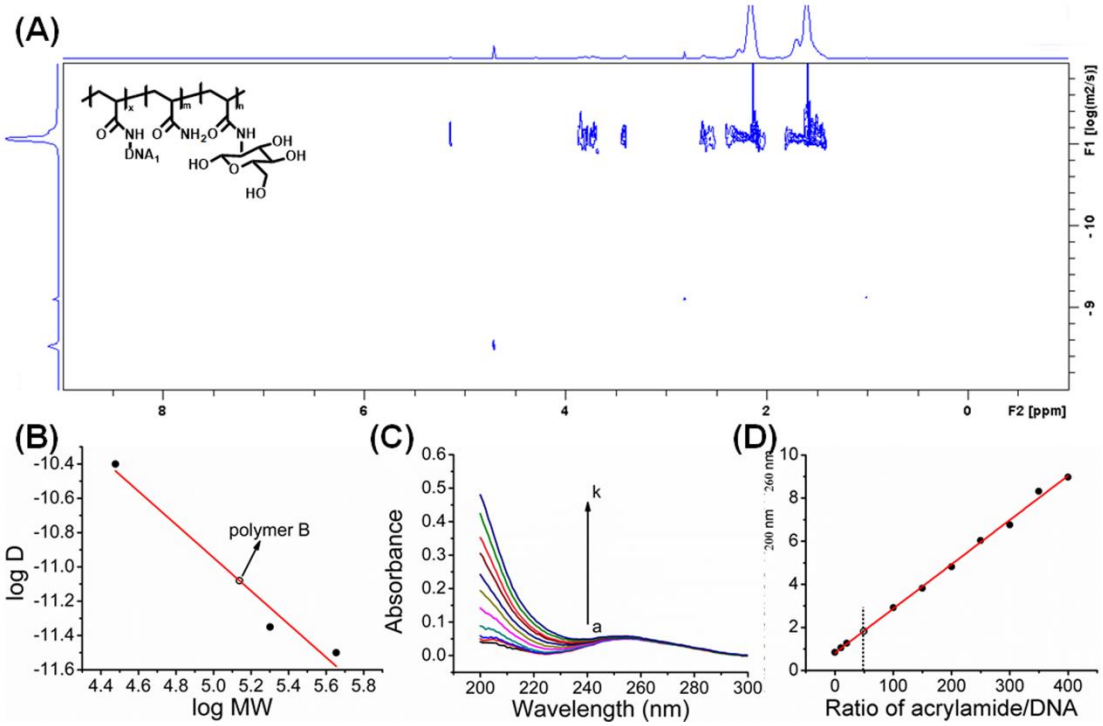

Fig. S2. (A) The DOSY spectrum of glucosamine and guanosine-rich nucleic acid tethers (**1**)-functionalized polyacrylamide chains (P_B). (B) Calibration curve corresponding to the Log/Log display of the diffusion coefficients vs. molecular weights of a series of polyacrylate derivatives. The point marked with an arrow corresponds to the diffusion coefficient of P_B extracted from its DOSY spectrum, resulting in an estimated average molecular weight of 137 000 (P_B). (C) Determination of the loading of acrylamide monomer by the nucleic acid tethers (**1**) of P_B: Absorption spectra of different concentrations of polyacrylamide in the presence of a constant concentration of acrydite modified nucleic acid, corresponding to 0.5 $\times 10^{-6}$ M: (a) 0, (b) 0.5 $\times 10^{-5}$ M, (c) 1 $\times 10^{-5}$ M, (d) 2.5 $\times 10^{-5}$ M, (e) 5 $\times 10^{-5}$ M, (f) 7.5 $\times 10^{-5}$ M, (g) 1.0 $\times 10^{-4}$ M, (h) 1.25 $\times 10^{-4}$ M, (i) 1.5 $\times 10^{-4}$ M, (j) 1.75 $\times 10^{-4}$ M, (k) 2.0 $\times 10^{-4}$ M, (l) 2.25 $\times 10^{-4}$ M, (k) 2.5 $\times 10^{-4}$ M. (D) Calibration curve corresponding to the absorbance ratio $A_{\lambda 200nm}/A_{\lambda 260nm}$ as a function of

acrylamide/DNA ratio. Dashed line indicates the loading of the DNA on the acrylamide copolymer, corresponding to acrydite modified nucleic acid tether (**1**):acrylamide = 1:49.

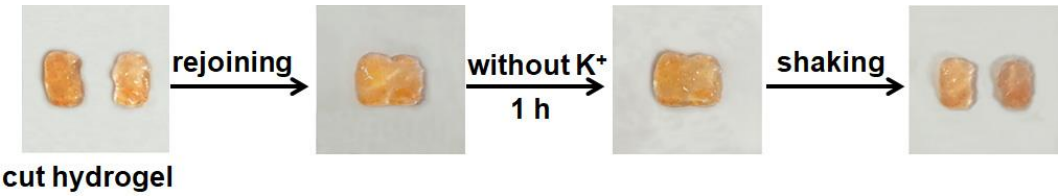

Fig. S3. The control experiment corresponding to the self-healing process: Self-healing steps associated with the two pieces of polyacrylamide hydogels crosslinked by the glucose-boronate ester crosslinking units and including the G-rich tethers in the absence of added K^+-ions.

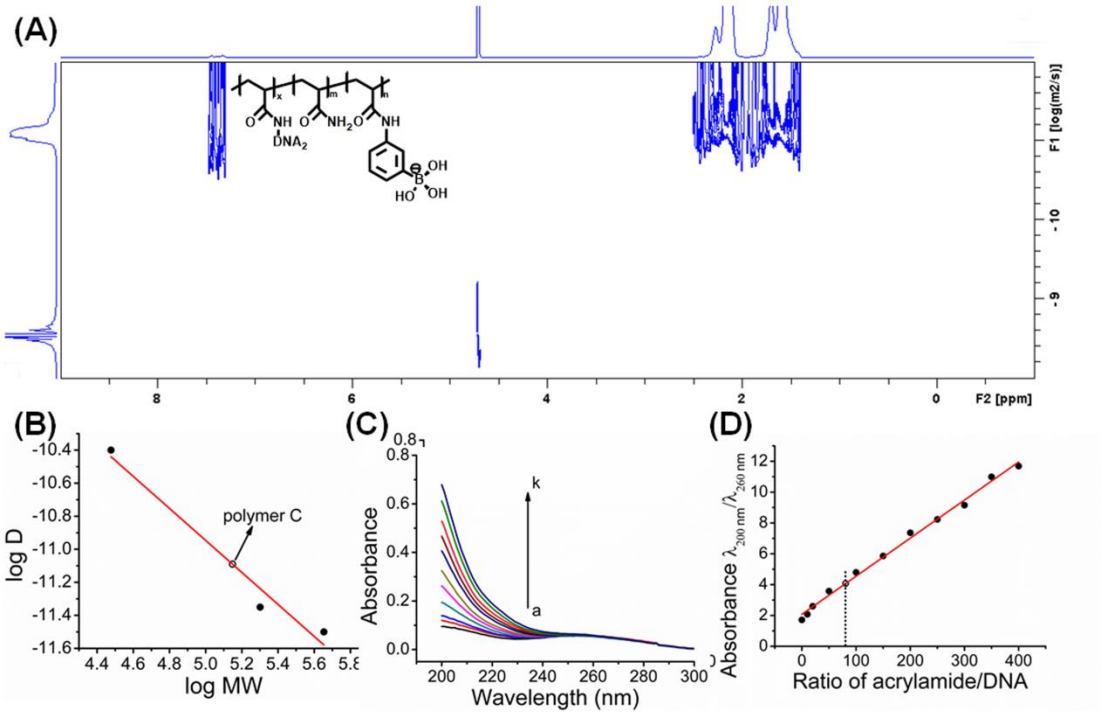

Fig. S4. (A) The DOSY spectrum of phenylboronic acid and nucleic acid tethers (**2**)-functionalized polyacrylamide chains (P_C). (B) Calibration curve corresponding to the Log/Log display of the diffusion coefficients vs. molecular weights of a series of polyacrylate derivatives. The point marked with an arrow corresponds to the diffusion coefficient of P_C extracted from its DOSY spectrum, resulting in an estimated average molecular weight of 140 000 (P_C). (C) Determination of the loading of acrylamide monomer by the nucleic acid tethers (**2**) of P_C: Absorption spectra of different concentrations of polyacrylamide in the presence of a constant concentration of acrydite modified nucleic acid, corresponding to 0.5 $\times 10^{-6}$ M: (a) 0, (b) 0.5 $\times 10^{-5}$ M, (c) 1 $\times 10^{-5}$ M, (d) 2.5 $\times 10^{-5}$ M, (e) 5 $\times 10^{-5}$ M, (f) 7.5 $\times 10^{-5}$ M, (g) 1.0 $\times 10^{-4}$ M, (h) 1.25 $\times 10^{-4}$ M, (i) 1.5 $\times 10^{-4}$ M, (j) 1.75 $\times 10^{-4}$ M, (k) 2.0 $\times 10^{-4}$ M, (l) 2.25 $\times 10^{-4}$ M, (k) 2.5 $\times 10^{-4}$ M. (D) Calibration curve corresponding to the absorbance ratio $A_{\lambda 200nm}/A_{\lambda 260nm}$ as a function of acrylamide/DNA ratio. Dashed line indicates the

loading of the DNA on the acrylamide copolymer, corresponding to acrydite modified nucleic acid tether (**2**):acrylamide = 1:81.

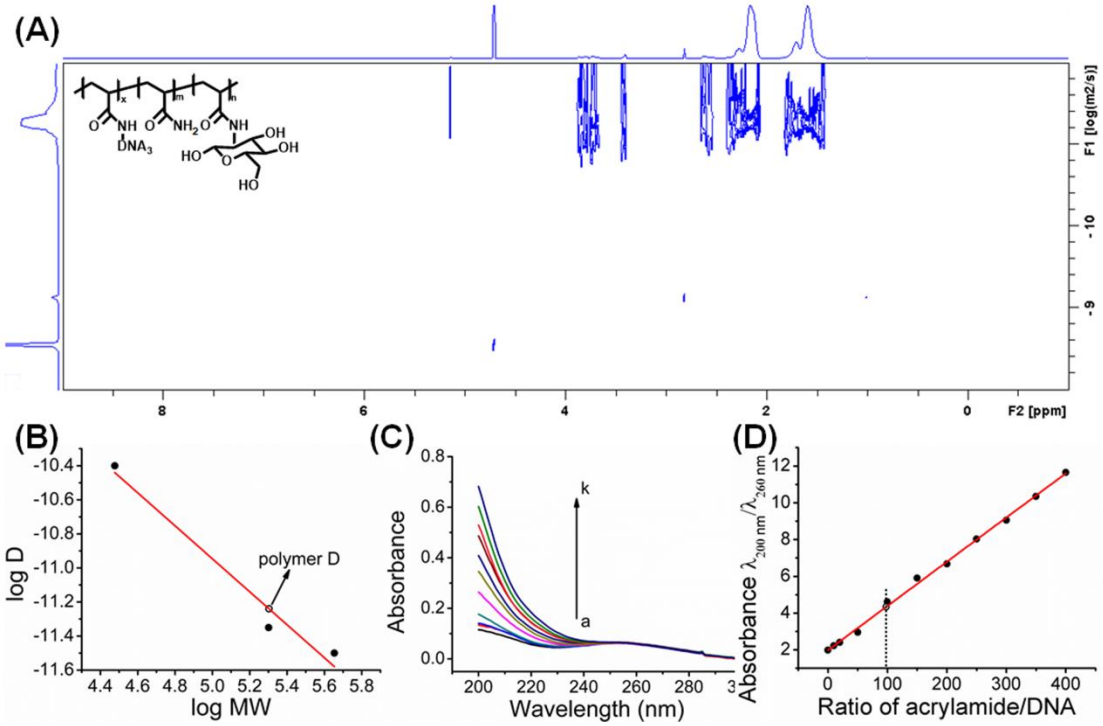

Fig. S5. (A) The DOSY spectrum of phenylboronic acid and nucleic acid tethers (**3**)-functionalized polyacrylamide chains (P_D). (B) Calibration curve corresponding to the Log/Log display of the diffusion coefficients vs. molecular weights of a series of polyacrylate derivatives. The point marked with an arrow corresponds to the diffusion coefficient of P_D extracted from its DOSY spectrum, resulting in an estimated average molecular weight of 200 000 (PD). (C) Determination of the loading of acrylamide monomer by the nucleic acid tethers (**3**) of P_D: Absorption spectra of different concentrations of polyacrylamide in the presence of a constant concentration of acrydite modified nucleic acid, corresponding to 0.5 $\times 10^{-6}$ M: (a) 0, (b) 0.5 $\times 10^{-5}$ M, (c) 1 $\times 10^{-5}$ M, (d) 2.5 $\times 10^{-5}$ M, (e) 5 $\times 10^{-5}$ M, (f) 7.5 $\times 10^{-5}$ M, (g) 1.0 $\times 10^{-4}$ M, (h) 1.25 $\times 10^{-4}$ M, (i) 1.5 $\times 10^{-4}$ M, (j) 1.75 $\times 10^{-4}$ M, (k) 2.0 $\times 10^{-4}$ M, (l) 2.25 $\times 10^{-4}$ M, (k) 2.5 $\times 10^{-4}$ M. (D) Calibration curve corresponding to the absorbance ratio $A_{\lambda 200nm}/A_{\lambda 260nm}$ as a function of acrylamide/DNA ratio. Dashed line indicates the

loading of the DNA on the acrylamide copolymer, corresponding to acrydite modified nucleic acid tether (**3**):acrylamide = 1:98.

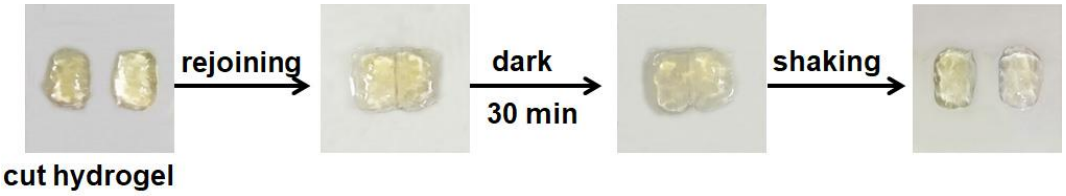

Fig. S6. The control experiment corresponding to the self-healing process: Self-healing of steps associated with the two pieces of polyacrylamide hydrogels crosslinked by the glucose-boronate ester crosslinking units and the photoresponsive duplex units (**2**)-(**4**) and (**3**)-*c*-azo (**5**) without visible light irradiation.

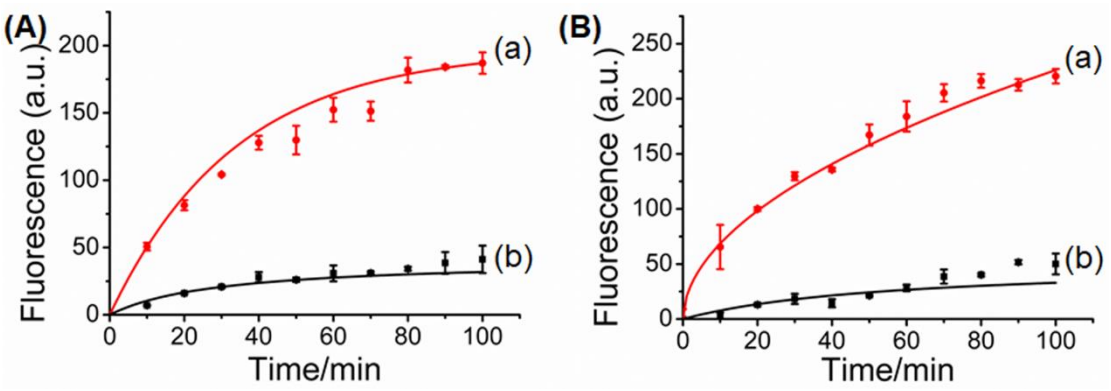

Fig. S7. The release profile of doxorubicin from the boronate ester-glucosamine/K^+-ion stabilized G-quadruplex crosslinked hydrogel (A) first cycle of drug loaded hydrogel and (B) second cycle of regenerated drug loaded matrices: (a) in the presence of 18-crown-6-ether, 10 mM, (b) without addition of the crown ether.

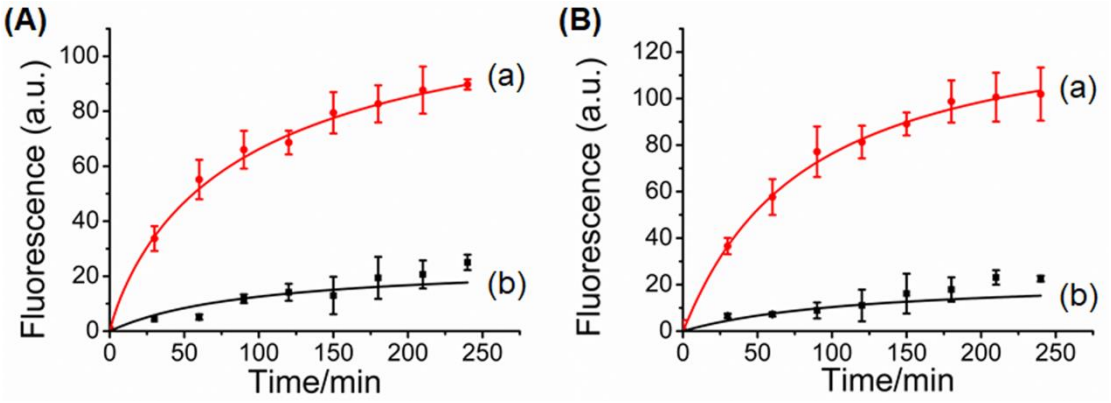

Fig. S8. The release profile of doxorubicin from the boronate ester-glucosamine/(2)-(4)/(3)-*t-azo* (5) photoresponsive duplexes crosslinked hydrogel (A) first cycle of drug loaded hydrogel and (B) second cycle of regenerated drug loaded matrices: (a) in the presence of UV irradiation, λ = 365 nm, (b) without UV irradiation.