Supplementary Information

Reversible Ag^+-Crosslinked DNA Hydrogels

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Experimental Section

Materials: Phosphate buffer, sodium nitrate, magnesium nitrate, cysteamine hydrochloride, silver nitrate, ammonium persulfate (APS), N,N,N',N'-Tetramethylethylenediamine (TEMED) and acrylamide solution (40%) were purchased from Sigma-Aldrich. Desalted DNA oligonucleotides were purchased from Integrated DNA Technologies Inc (Coralville, IA, DNA). The sequences are detailed in Table 1. All the solutions were prepared with ultrapure water purified by a NANOpure Diamond system (Barnstead International, Dubuque, IA, USA).

Table 1. Sequences of the DNA strands

(**1**)	5'-TATTATACTTTACAACATGAAAAGATTGGATATAGTATAAGGAT-3'
(**2**)	5'-TATTATACTTTACATACAGAGTCAGGTGCAATCTTTTCATGT-3'
(**3**)	5'-TATTATACTTTACAATCCTTATACTATATCCACCTGACTCTGTA-3'
(**4**)	5'-TCTAAACTATAATAACTAGATACATACAG-3'
(**5**)	5'-TCTAAACTATAATACTGTATGTATCTAGT-3'
(**6**)	5'-Acrydite – AATCTTAACATT-3'

Synthesis of the acrylamide/acrydite nucleic acid copolymer

A buffer solution (Tris-HCl, 10 mM, pH = 8.0), 200 μL, that included the acrydite nucleic acid, (**6**), 1 mM, and 3% acrylamide was prepared. Nitrogen was bubbled through the solution. Subsequently, 2.8 μL of a 0.5 mL aqueous solution that included 50 mg APS and 25 μL TEMED, was added to the monomer mixture. The resulting

mixture was allowed to polymerize at room temperature for five minutes, and then the mixture was further polymerized at 4˚C for 12 hours. The resulting polymer was purified from unreacted monomer units, salts and the initiator, using a Microcon (Millipore) spin filter unit (MWCO 10 kD). The purified polymer was removed from the filter and it was dissolved in 200 μL of water. The ratio of acrylamide : acrydite nucleic acid was determined spectroscopically.

Preparation of the different hydrogels and their switchable transitions between hydrogel-solution states.

For the preparation of the crosslinked Y-shaped DNA hydrogel according to Fig. 1, solutions of the Y-shaped subunits (**1**)/(**2**)/(**3**) and of the duplex structures (**4**)/(**5**) were prepared. Separate buffer solutions (10 mM phosphate, 400 mM sodium nitrate, 10 mM magnesium nitrate, pH = 7.0) were added to the strands (**1**), (**2**) and (**3**), 0.6 mM each, or the strands (**4**) and (**5**), 0.9 mM each, respectively. Each of these mixtures was heated to 85˚C, to separate any undesired hybridization products, and the solutions were allowed to cool down to room temperature for two hours, to yield solutions consisting of the hybridized (**1**)/(**2**)/(**3**) units or the (**4**)/(**5**) units. For the preparation of the hydrogel, equal volumes of the solution of the (**1**)/(**2**)/(**3**) Y-shaped units and the solution of (**4**)/(**5**) duplex structures were mixed. $AgNO_3$, 1.8 mM, was added to the resulting mixture solution composed of 0.3 mM (**1**)/(**2**)/(**3**) Y-shaped units and 0.45 mM (**4**)/(**5**) duplex structures. The hydrogelation occurred within five minutes. For the dissolution of the hydrogel, cysteamine was added to the hydrogel to

yield a final concentration of 1.8 mM. The dissolution of the hydrogel occurred within ca. five minutes.

For the preparation of the acrylamide/acrydite nucleic acid (**6**) hydrogel the copolymer acrylamide/acrydite nucleic acid (**6**) was dissolved in a buffer solution (10 mM phosphate, 400 mM sodium nitrate, 10 mM magnesium nitrate, pH = 7.0) to yield a copolymer solution based on the nucleic acid (**6**) tethers (0.4 mM). $AgNO_3$, 0.4 mM, was added to the copolymer mixture to induce gelation. The gelation was completed within five minutes. For the separation of the hydrogel a minute volume of concentrated cysteamine solution that yields a concentration of 0.4 mM in the gel was added. The separation of the hydrogel occurred within ca. five minutes.

Rheology: The in-situ hydrogel formation, mechanical properties, and cross-linking kinetics were characterized by an AR-G2 rheometer (TA Instruments). Time-sweep oscillatory tests were performed with a 20 mm parallel-plate geometry, using 150 μL of a fresh solution (resulting in a gap size of 0.35 mm), while decreasing the temperature from 60˚C to 20˚C immediately at the beginning of the measurement. Oscillatory strain (0.01-100%) and frequency sweeps (0.01-100 Hz) were conducted 15 min after adding the Ag^+, in order to find the linear viscoelastic region, at which the time sweep oscillatory tests were performed. The linear viscoelastic region for the Y-shape DNA hydrogel system was found to be at 1% strain and 1 Hz frequency, while for the acrylamide hydrogel system it was found to be at 1% strain and 0.8 Hz frequency.

Microscopy: SEM images were taken with Extra High Resolution Scanning Electron Microscope Magellan (TM) 400L, microscope Settings: 2 kV, 6.3 pA. Slides (Si) were first washed with distilled water followed by ethanol and acetone, then UV / ozone cleaned using a T1O×10 / OES / E UV/ozone chamber from UVOCS, Inc. (USA). Subsequently, the slides were incubated in 2% aminopropyltriethoxysilane for 30 minutes, and heated to 110˚C for 10 minutes to generate an amino monolayer. A piece of freshly formed hydrogel was placed on the slide surface. Then the hydrogel sample was frozen by immersing it in liquid nitrogen. The frozen hydrogel sample was dried by sublimation of the formed ice under high vacuum, and further metal-coated with Au/Pd.

Elimination of the Ag^+-Cysteamine complex from the hydrogel and the recovery of the mixture of components comprising the hydrogel.

The resulting Ag^+-Cysteamine complex generated upon the formation/dissociation cycles of the hydrogels was eliminated by applying a Microcon spin filter unit ((MWCO 10 kD)). Before the purification procedure, excess of cysteamine was added to ensure that all the Ag^+ ions were bound to cysteamine. The purified hydrogel components were removed from the filter unit and were dissolved in a buffer solution (10 mM phosphate, 400 mM sodium nitrate, 10 mM magnesium nitrate, pH = 7.0).

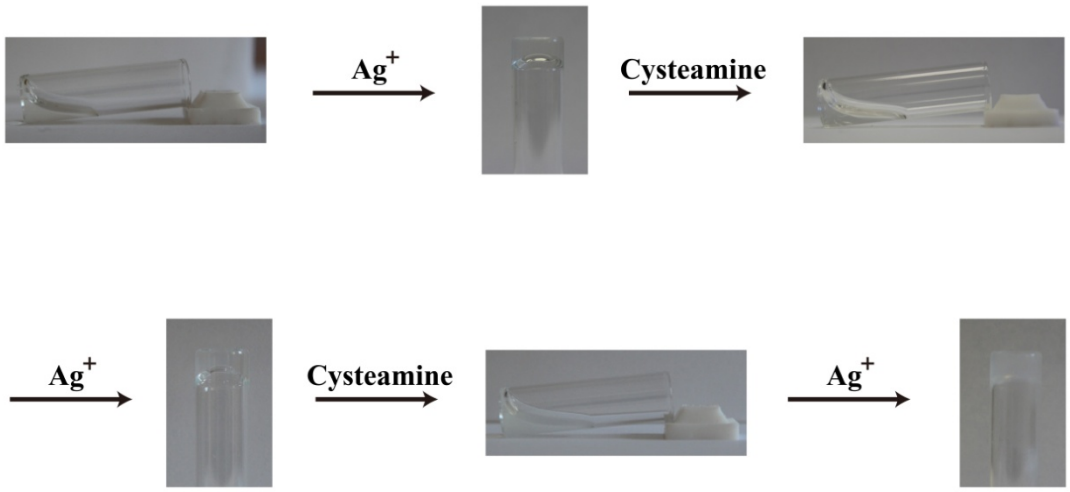

Fig. S1 Images corresponding to switchable and reversible Ag^{+}-stimulated hydrogelation of the Y-shaped DNA subunits, and the transition of the hydrogel into a solution according to Fig. 1.

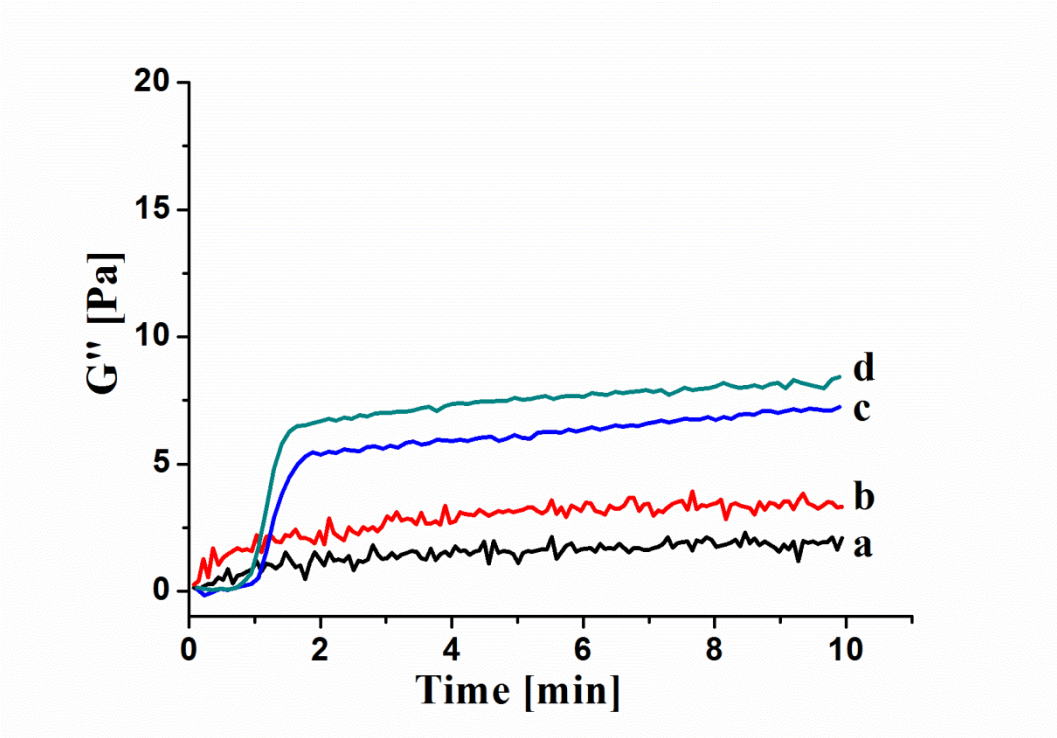

Fig. S2 Time-dependent changes of the loss modulus G" corresponding to systems where the Y-shaped units, 0.3 mM, and 0.45 mM of the duplex DNA subjected to variable concentrations of Ag^{+}: (a) 0 mM, (b) 0.36 mM, (c) 1.35 mM and (d) 1.8 mM.

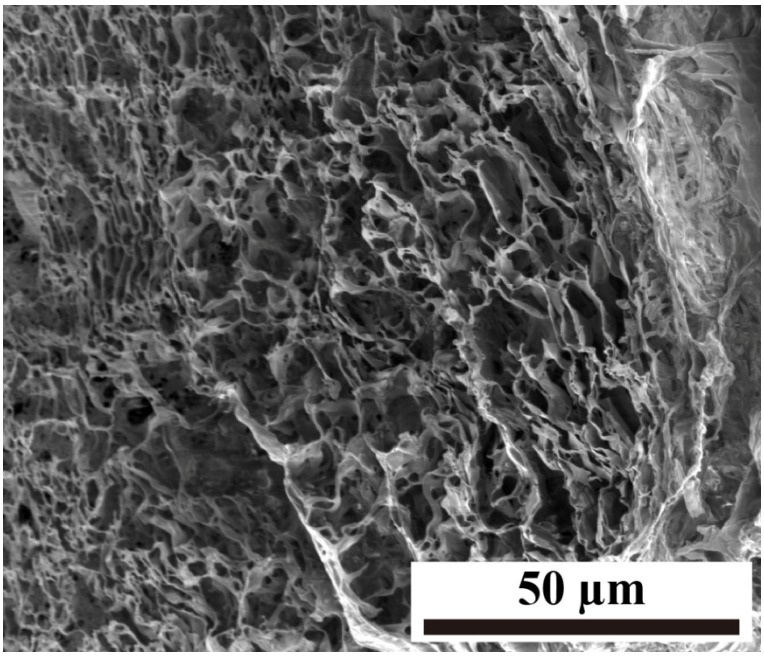

Fig. S3 SEM image of the freeze-dried hydrogel consisting of the Ag^{+}-crosslinked Y-shaped DNA subunits, according to Fig. 1.

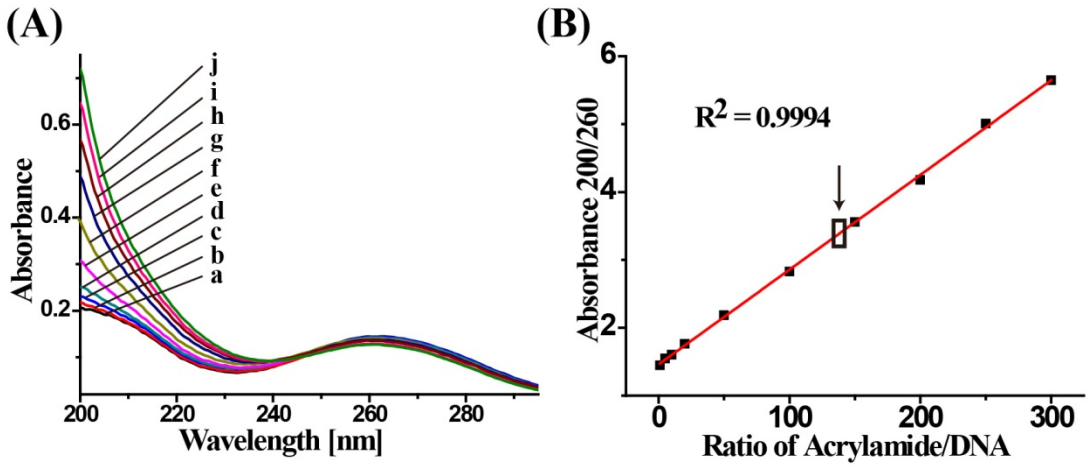

Fig. S4 Spectroscopic characterization of the ratio of acrylamide:acrydite nucleic acid in the copolymer chain. (A) Absorption spectra corresponding to: (a) the free acrydite-nucleic acid (6), 1 x 10^{-6} M. (b) to (j) upon addition of pure acrylamide chains that include total monomer concentration corresponding to: (b) 5 x 10^{-6} M; (c) 1 x 10^{-5} M; (d) 2 x 10^{-5} M; (e) 5 x 10^{-5} M; (f) 1 x 10^{-4} M; (g) 1.5 x 10^{-4} M; (h) 2 x 10^{-4} M; (i) 2.5 x 10^{-4} M; (j) 3 x 10^{-4} M. (B) Derived calibration curve. Square marked with arrow corresponds to the absorbance features of the resulting acrylamide/acrydite nucleic acid (**6**) copolymer system in this study.