Supplementary Information (SI) for Soft Matter.
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Supporting information

A Cofactor Mediated Supramolecular Oligo-Adenine Triplex for Reprogrammable Macroscopic Hydrogel Assembly

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Materials. Cyanuric acid (CA), thiazole orange (TO), deoxyribonucleic acid sodium salt from salmon testes (DNAss), magnesium chloride, ammonium persulfate (APS), *N,N,N′,N′*-tetramethylethylenediamine (TEMED), acrylamide/bis-acrylamide (29:1, 40 %) and other chemicals were purchased from Sigma-Aldrich, unless otherwise noted. Acrylamide-polyethylene glycol-azide (ACA-PEG-N_3, 2 k) was purchased from Biopharma PEG Scientific Inc. GelRed was purchased from Biotium. DAPI, SYBR Safe, and SYBR Gold were purchased from Thermo Fisher Scientific. 5′-terminal dibenzocyclooctyne (DBCO) modified A21 strands were purchased from Integrated DNA Technologies Inc. (Coralville, IA). 5′-terminal pyrene modified A21 strands were purchased from Gaia Science Pte. Ltd. Ultrapure water (18.2 MΩ·cm) purified by a Milli-Q system was used to prepare the solutions. The sequences of DNA strands used in this study are:

A21: 5'-AAA AAA AAA AAA AAA AAA AAA-3'
DBCO-A21: 5'-DBCO-AAA AAA AAA AAA AAA AAA AAA-3'
Py-A21: 5'-pyrene-AAA AAA AAA AAA AAA AAA AAA-3'

UV–vis and transmittance spectra. The UV–vis and transmittance spectra of samples were collected with Cary 3500 Compact Peltier UV–Vis Spectrophotometer (Agilent).

CD spectra. The CD spectra of A21 (5 μM) in the absence or presence of CA cofactors (15 mM) were recorded in 1×PBS buffer (pH 5 or pH 8) containing 10 mM $MgCl_2$ by JASCO J-815CD Spectrometer.

Fluorescence measurement. The fluorescence of TO (10 μM) was measured with A21 (5 μM) in the presence or absence of CA cofactors (10 mM) in 1×PBS buffer (pH 5 or pH 8) containing 10 mM $MgCl_2$. For the control experiments, the same concentrations of samples as above were adopted. The excitation wavelength for TO was 482 nm, and the spectrum was recorded in the wavelength range of 500–700 nm. The fluorescence of pyrene was measured with Py-A21 (5 μM) in the presence or absence of CA cofactors (15 mM) in 1×PBS buffer (pH 5 or pH 8) containing 10 mM $MgCl_2$. The excitation wavelength for pyrene was 349 nm, and the spectrum was recorded in the wavelength range of 370–600 nm. The measurements were conducted by fluorescence spectrophotometer (Hitachi, Model F-7100, Japan).

SEM imaging. SEM images were obtained with a field emission scanning electron microscope (JEOL JSM-6700F, Japan). According to the procedures, the hydrogel cubes were prepared, freeze-dried, and then coated with an Au/Pd film before imaging.

Rheology measurements. The mechanical properties of the hydrogel cubes were characterized by an HAAKE™ MARS™ Rheometer (Thermo Scientific). The samples were placed onto a parallel-plate geometry (35 mm in diameter) at a temperature of 25 °C.

Preparation of A21 strands modified hydrogel cubes. To prepare hydrogel cubes, a mixture of acrylamide/bis-acrylamide (29:1, 10 % v/v), ACA-PEG-N_3 (5 % w/v), and DNAss (0.02 % w/v) in 0.5×TBE buffer underwent copolymerization by using APS/TEMED as initiator/accelerator in custom cube molds (5×5×5 mm), yielding hydrogel cubes with azide

functional groups. Subsequently, the synthesized hydrogel cubes were extracted from the molds, thoroughly washed, and dialyzed in 1×PBS buffer (pH 7, containing 10 mM $MgCl_2$) for three days to eliminate unpolymerized monomer units and initiators. The hydrogel cubes were further subjected to staining with DNA dyes (5 μL in 400 μL 1×PBS buffer), including DAPI, GelRed, SYBR Safe and SYBR Gold. Following an overnight staining period (12 h), the hydrogel cubes were removed from the stains and rinsed three times with 1×PBS buffer. After staining, DBCO-A21 strands were linked to azide modified hydrogel cubes via SPAAC by incubating them in the strand solutions (20 μM, in 250 μL 1×PBS buffer) for overnight (12 h). The A21-modified cubes were then washed with 1×PBS buffer. The average quantity of A21 strands attached to each cube was determined by measuring the A21 amount before and after the incubation in solution (~4.5 μM). The difference in amount reflected the desired A21 modification on the cube (~3.9 nmol).

Assembly and disassembly of hydrogel cubes. The A21 modified hydrogel cubes existed as individuals in 1×PBS buffer (pH 8, containing 10 mM $MgCl_2$) in a petri dish. To facilitate LEGO-style assembly, the buffer was first removed, and the cubes were configured into a square architecture with face-to-face alignments. Subsequently, CA cofactor solution (10 mM in 1×PBS buffer, pH 5, containing 10 mM $MgCl_2$) was applied to the interfaces between these cubes and allowed to sit overnight, achieving assembly crosslinked by A-CA triplexes between hydrogel cubes. Following the assembly, additional CA cofactor solution (10 mM in 1×PBS buffer, pH 5, containing 10 mM $MgCl_2$) was introduced into the petri dish to submerge the assembled square construct bridged by A-CA triplexes. For the disassembly, the CA cofactor solution (pH 5) was replaced by a CA solution of pH 8, which deprotonated CA cofactors, resulting in the dissociation of hydrogel cubes into individuals. Similar processes were adopted to achieve the recycled assembly and disassembly of hydrogel cubes. pH indicator papers were inserted into the petri dish to show the pH values.

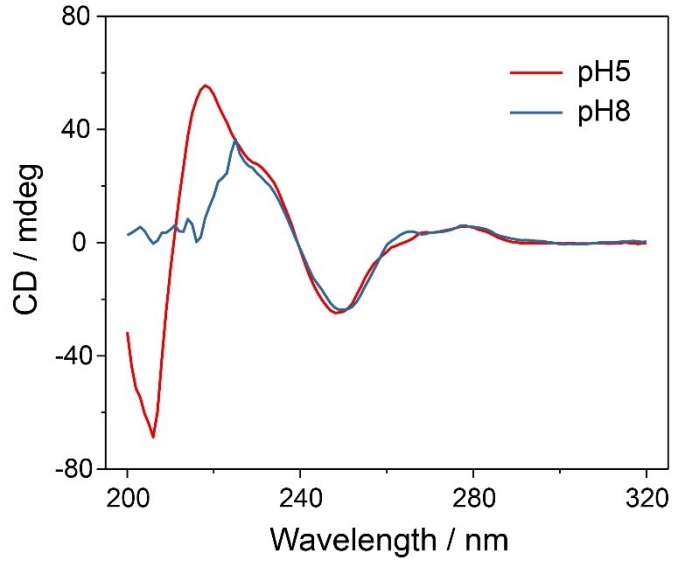

Figure S1. CD spectra of A21 strands at pH 5 and pH 8.

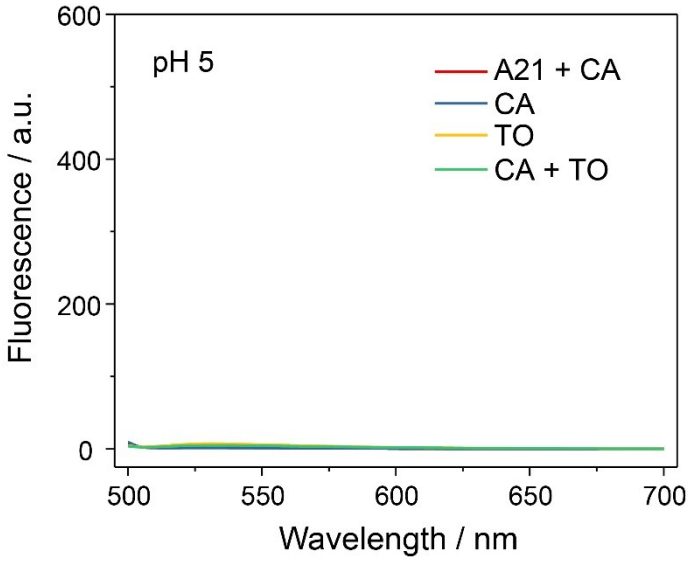

Figure S2. Fluorescence spectra of A21 with CA, CA, TO, and the mixture of CA with TO at pH 5.

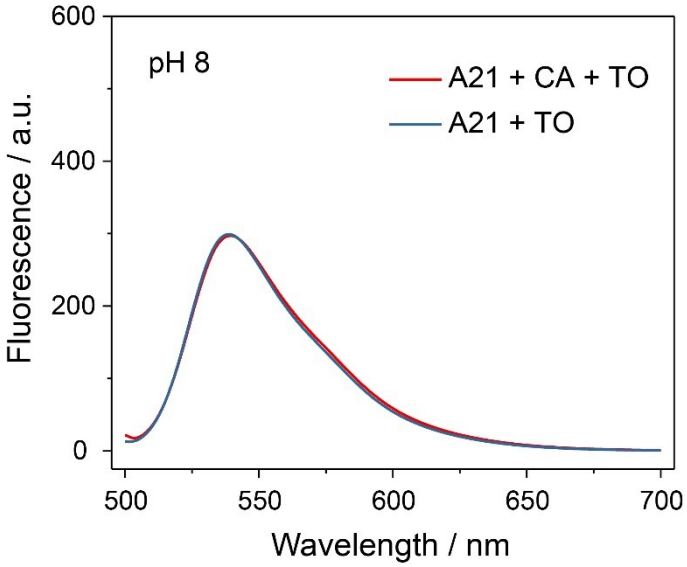

Figure S3. Fluorescence spectra of TO with A21 strands in the presence and absence of CA cofactors at pH 8.

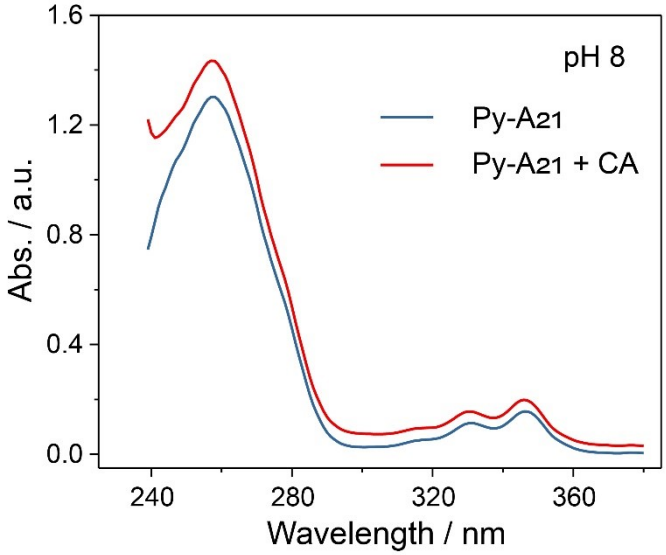

Figure S4. Absorbance spectra Py-A21 strands in the absence and presence of CA cofactors at pH 8.

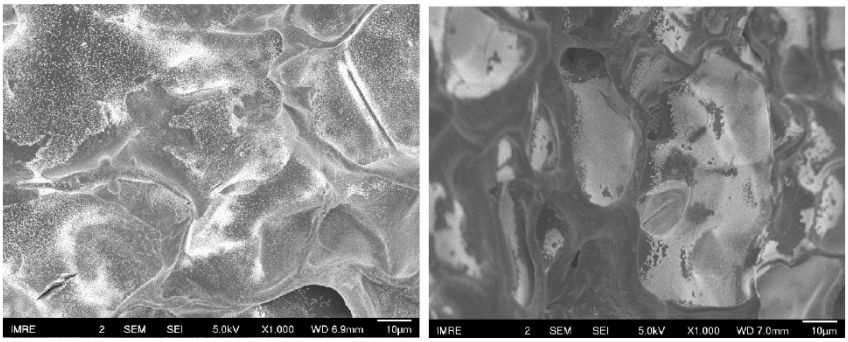

Figure S5. SEM images of hydrogel cube before (left) and after (right) the modification with DBCO-A21 strands.

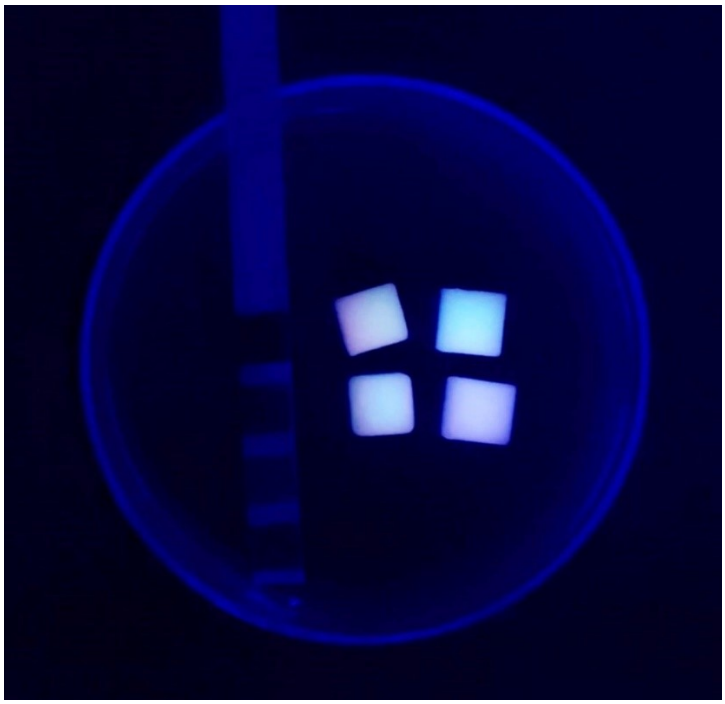

Figure S6. Enlarged photo of panel vi in Figure 5B.