Supplementary Materials for

DNA sequence–directed shape change of photopatterned hydrogels via high-degree swelling

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**Supplementary Materials:**

**Materials and Methods:**

**DNA Sequences and Sequence Design.** All oligonucleotide sequences are listed in Table S1. The sequences for system 1 crosslinks and polymerizing hairpins, H1 and H2, are based on those used by Venkataraman et al (20). Oligonucleotides were supplied by Integrated DNA Technologies (IDT) in their lyophilized form. Sequences for all terminating hairpins and the sequences for the crosslinks and hairpins in systems 2-4 were designed using the DNA design package, available at: www.dna.caltech.edu/DNAdesign/. This program produces sequences with the necessary complementarity to form the desired secondary structures, while minimizing other potential interactions (31).

**Preparation of DNA-crosslinked and N,N’-methylenebis(acrylamide) (BIS)-crosslinked pregel solutions.** Both sets of gels were prepared by UV-initiated, free radical copolymerization of acrylamide along with either N,N’-methylenebis(acrylamide) (BIS) or a DNA duplex as the crosslinker (Fig. S1). To enable its copolymerization with acrylamide, the pre-annealed DNA duplex crosslinker contained oligonucleotides modified at the 5’ end with an acrydite moiety. For the DNA pregel solution, stock solutions of the crosslink strands C and C’ (which contain acrydite modifications), or the crosslink strands for the corresponding system, were first prepared by resuspending lyophilized DNA samples to a final concentration of approximately 25 mM in TAE buffer (40 mM tris-acetate, 1 mM EDTA) that had been previously diluted from 50x stock (Life Technologies, Catalog #24710-030) and supplemented with 12.5 mM magnesium acetate tetrahydrate (Sigma #228648), herein referred to as TAE/Mg$_2^{2+}$. Oligonucleotide concentrations were verified by absorbance spectroscopy at 260 nm. To prepare the DNA copolymer gel, referred to as poly(Am-co-DNA), TAE/Mg$_2^{2+}$/Ca$_2^{2+}$– herein referred to as TAE/Mg$_2^{2+}$/Ca$_2^{2+}$– and MilliQ water were added to the crosslink strands. The calcium chloride was added to prevent premature dissolution of the poly(acrylic acid) (PAA) sacrificial layer. This DNA crosslinks were then annealed by incubating the solution at 90 °C for five minutes, followed by cooling the solution from 90 °C to 20 °C at 1 °C per minute to allow crosslinks to hybridize. Immediately before photopolymerization, acrylamide (Bio-Rad Catalog #161-0100), Irgacure 2100 (Ciba), and, if applicable, methacryloxyethyl thiocarbamoyl rhodamine B (Polysciences, Inc., catalog #23591) were added to the solution. In cases where the gels were stained with SYBR Green I nucleic acid stain (Invitrogen catalog #S7563), the rhodamine B monomer was omitted. The solution was then mixed via pipet and degassed under vacuum for 5 minutes to minimize the effect of O$_2$ on radical chain polymerization. The final concentrations of all pregel components are as follows: 1.154 mM of strands C and C’, 1x TAE/Mg$_2^{2+}$ buffer, 11.1 mM calcium chloride, 1.41 M acrylamide, 3 vol% of Irgacure 2100, and, if applicable, 2.74 mM methacrylated rhodamine B.

The BIS-crosslinked gel solutions were prepared by mixing MilliQ water, TAE/Mg$_2^{2+}$/Ca$_2^{2+}$ buffer, 40% (w/v) 19:1 Am:BIS (BioRad Laboratories, Inc., catalog #1610144), 50% (v/v) Irgacure 2100 in 1-butanol, and, if applicable, 50 mM fluorescein-O-methacrylate (Sigma, catalog #568864) into a test tube. The final concentrations of TAE/Mg$_2^{2+}$ buffer, and calcium chloride are the same as in the poly(Am-co-DNA) pregel solution. The final concentrations of the other species are as follows: 5% (w/v) of 19:1 Am:BIS, 1.5% (v/v) Irgacure 2100, and, if
applicable, 2.74 mM fluorescein-O-methacrylate. The BIS and acrylamide pregel solution was then mixed and degassed following the same protocol of the DNA pregel solution.

**Photolithography chamber preparation.** The photolithography chambers were prepared according to a previously published protocol (32). The bottom glass slide served as a substrate onto which the hydrogel samples adhered after photopatterning, while the top slide served as a chromium (Cr) photomask to selectively expose regions of the pregel solution to ultraviolet (UV) light and initiate radical chain polymerization. The top slide of the photolithography chamber was prepared by spin coating SC 1827 (Microposit S1800 Series) on a clean glass slide at 3500 rpm for 3 minutes, followed by baking at 115 °C for 60 seconds. The coated slides were irradiated with a 317 mJ/cm² dose of 365 nm UV light through film masks designed using AutoCAD and printed by Fineline Imaging. After UV exposure, the glass slides were developed with a 1:10 (w/w) solution of Microposit 351 Developer (Shipley) and DI water, and were dried with N₂ gas. Next, a 200 nm layer of Cr was deposited on the glass slide by physical vapor deposition (PVD), after which the slides were consecutively rinsed with acetone and isopropyl alcohol, and were dried under N₂ gas to remove the unexposed regions of 1827 positive photoresist. Once prepared, the Cr mask was spin-coated with CYTOP (Type M, Bellex International Corp.) at 4000 rpm and baked at 90 ºC for 2 hours to ensure evaporation of the organic solvent. The CYTOP-coated chromium mask prevented the DNA gel from sticking to the mask and allowed for minimal edge roughness.

The bottom slides of the photolithography chamber (Catalog #16004-424, VWR) were prepared by sonicating in 10% (w/w) NaOH for 30 minutes, rinsing with MilliQ water, and drying under N₂ gas. The bottom slide was then treated with O₂ plasma for 5 minutes to fully oxidize the glass surface. Next, a single layer of polyimide tape (~60 µm thick) was placed along the width of the glass slide to act as a spacer. For thinner hydrogels, aluminum foil (~14 µm thick) was used as a spacer. A roughly 200 nm thick layer of 5% (w/w) PAA crosslinked with calcium was then deposited onto the substrate according to a previously reported protocol (33). Additional washing (3 min in DI water) and baking steps (5 min at 150°C) were added to the protocol to remove calcium salt deposits present on the substrate after crosslinking the PAA in a solution of CaCl₂. The final photolithography chamber was assembled by clipping the top Cr mask and bottom PAA-covered substrate together with binder clips (Office Depot). The chrome layer of the mask faced inward and came into direct contact with the pregel solution.

**Photopatterning of poly(Am-co-DNA) monolayer and poly(Am-co-BIS)/poly(Am-co-DNA) bilayers.** To photopattern DNA hydrogel monolayers, the DNA pregel solution was injected via pipet into the photolithography chamber. The chamber was then exposed to 365 nm UV light (Neutronix Quintel aligner) for a total light dose of 240 mJ/cm² as determined by multiplying the measured UV intensity (Vari-Wave II, 365 nm sensor; Quintel) by the exposure time. The chamber was then gently disassembled and 1 mL of 1 M NaCl was aliquoted onto the substrate to dissolve the PAA sacrificial layer and yield freestanding samples. The monolayers were then placed into a PDMS-coated polystyrene dish to which approximately 2 mL of TAE/Mg²⁺ was added.

To prepare the first hydrogel layer of a bilayer structure, the previous photopatterning protocol was followed using Am-co-BIS-co-fluorescein pregel solution except that after UV exposure the substrate was washed with approximately 200 µL of TAE/Mg²⁺/Ca²⁺ to remove unreacted pregel
solution from the patterned structures. The UV dose for all BIS-crosslinked hydrogel structures is approximately 280 mJ/cm². The first gel layer was then allowed to dry at room temperature for approximately 20 minutes. The second layer of the hydrogel bilayer structures was fabricated using Am-co-DNA-co-rhodamine pregel solution. Prior to photopatterning the second gel layer, an additional layer of polyimide tape was placed on the substrate and the second Cr mask was then aligned with the first gel layer using a mask aligner. After satisfactory alignment was achieved, the Am-co-DNA-co-rhodamine solution was injected via pipet into the photolithography chamber and exposed to UV light for a total dose of 240 mJ/cm². At this point, when the bilayer bar structures were fabricated, the chamber was gently disassembled and approximately 1 mL of 1 M NaCl was aliquoted onto the substrate to dissolve the sacrificial layer and yield freestanding bilayer bar structures (Fig. S12). When the petal or crab bilayer structures were fabricated, the above process of washing the patterned structures, aligning the masks, injecting pregel solution, and exposing to UV light is repeated until the final hydrogel domain is patterned, at which point the photolithography chamber is disassembled and 1 M NaCl was added to yield free-floating hydrogel structures.

**Quantifying the DNA-driven expansion and shape change of poly(Am-co-DNA) hydrogels.**

To measure the rate of expansion and final uniaxial swelling rate of poly(Am-co-DNA) hydrogels driven by DNA polymerization, Am-co-DNA pregel solution was prepared as previously described, without methacryloxyethyl thiocarbamoyl rhodamine B, and gels were patterned into either 60 µm or 14 µm thick, 1 x 1 mm square shapes using appropriate photomasks. After fabrication, the DNA gels were stained overnight in a solution of 2x SYBR Green I (Invitrogen catalog #S7563) and TAE/Mg²⁺ buffer. The SYBR staining solution was then removed and the samples were washed several times with TAE/Mg²⁺ buffer. After the last wash, 2 mL of fresh TAE/Mg²⁺ was added to the petri dish.

All DNA hairpin monomers were supplied by IDT in their lyophilized form, and were resuspended to a final concentration of 2 mM in TAE/Mg²⁺ buffer. All swelling and actuation experiments contained a final hairpin concentration (polymerizing hairpin monomer plus terminator hairpin monomer) of 20 µM. Prior to adding the hairpin solution to the DNA gel samples, the hairpin monomers were snap-cooled in order to remove any aggregates or polymers that may have formed by heating oligonucleotide solutions at 95 ºC for at least five minutes, followed by cooling on ice for 2 minutes. Next, the hairpin solution was gently added via syringe so as not to disturb the gel samples. Swelling was recorded via time-lapse fluorescence imaging using a gel imager (Syngene EF2 G:Box) equipped with a blue light transilluminator (Clare Chemical, emission max ~450 nm) and a UV032 filter (Syngene, bandpass 572-630 nm). Images were captured in 20 minute intervals until a steady-state was reached or the gels were no longer visible. The uniaxial swelling ratio of the samples was measured manually in either MATLAB or ImageJ. MATLAB code available upon request.

For DNA-driven expansion of the bilayer structures in hairpin solution (20 µM, 98% polymerizing monomers, 2% terminating monomers), the volumetric swelling ratios of the BIS and DNA gel domains within the bilayer beams were measured using a Nikon AZ100 multi-zoom epifluorescence microscope and Zeiss AxioObserver Yokogawa CSU-X1 spinning disc confocal microscope. The sizes of the domains of the 12 bilayer beams were characterized. Some of the bilayers lain on their sides, which allowed us to image them top-down and others side-on. We measured the contour lengths, widths and thicknesses of the BIS and DNA layers of all the
bilayers before and after adding DNA hairpins (20 µM solution composed of 98% polymerizing monomers and 2% terminating monomers) and averaged the values. The average volumetric swelling ratio was calculated by dividing the volume of the gels after and before adding DNA hairpins.

Images of bilayer, flower and crab structures were captured using a Nikon AZ100 multi-zoom epifluorescence microscope equipped with a Nikon DS-Fi1 camera. Fluorescence images of poly(Am-co-DNA-co-rhodamine) gel samples were captured using a Nikon B-2E/C filter cube (excitation 465-495 nm, bandpass emission filter 515-555 nm), whereas images for poly(Am-co-BIS-co-fluorescein) samples were captured using a Nikon G-2E/C filter cube (excitation filter 528-553 nm, bandpass emission filter 590-650 nm). Domains with different crosslink sequences (patterned in different sequence steps) were false colored in the fluorescence micrographs in Figure 4, and isolated devices are presented on a black background for clarity. MATLAB code available upon request.

**SEM Imaging of poly(Am-co-DNA) hydrogels.** Two 5 mm x 5 mm hydrogel samples, each 600 µm thick, were fabricated by photopolymerization of pregel solution within a PDMS micromold. The pregel solutions were prepared without methacryloyloxyethyl thiocarbamoyl rhodamine B as previously described. Prior to photopolymerization the PDMS mold was treated with air plasma for five minutes using a surface corona treater (Electro-Technic Products, model BD-20). The pregel solution was then aliquoted into sample wells of the PDMS mold and exposed to 365 nm UV light (Neutronix Quintel aligner) at an intensity of 7.55 mW/cm² and dose of 680 mJ/cm² to ensure complete curing of the pregel solution. The samples were then gently excised from the mold and placed into separate petri dishes containing 2 mL of fresh TAE/Mg²⁺ buffer. The gels were allowed to swell to equilibrium overnight via solvent uptake. To one DNA gel sample was added two 500 µL hairpin solutions, each containing 117.6 µM of the regular hairpin monomer and 2.4 µM of the respective terminator hairpin (e.g., 117.6 µM H₁ and 2.4 µM H₁T) for a final concentration of 19.6 µM and 0.4 µM of the polymerizing hairpin monomer and terminator monomer, respectively. One milliliter of TAE/Mg²⁺ was added to the other sample as a control. The two samples were left out at room temperature for two weeks, at which point the swelling solution was removed and the gels were frozen in liquid nitrogen for five minutes (34). The gel samples were then lyophilized for 24 hours to fully remove the swelling solution in preparation for SEM imaging (Labconco, Freezone Benchtop Freeze Dry System, Catalog #7382021).
Supplementary Text:

Finite Element Model of BIS/DNA Bilayer Actuation

Various theoretical hydrogel models have been developed in recent years to explain the coupled mechanical and stimuli-responsive swelling behavior of hydrogels and to support the design of active hydrogel structures (35, 36). We previously developed a constitutive theory for thermoresponsive hydrogels and showed that it can accurately predict the equilibrium configuration of pNIPAM gels and composite structures in response to temperature and mechanical stimuli (32, 37, 38). In our model, we neglected the kinetics of diffusion and assumed that the hydrogel remained in equilibrium throughout the deformation, which was justified by the short diffusion time permitted by micrometer-scale thickness of the bilayer structures.

For the constitutive model, we first defined a deformation field \( x = \varphi(X) \), that maps material points \( X \) in the initial undeformed dry polymer network configuration to spatial points \( x \) in the current deformed hydrogel configuration. The deformation gradient tensor is defined as \( F = \partial x / \partial X \) from the initial configuration to the current configuration. To model the stress-free swelling of the gel, the deformation gradient tensor \( F \) is further decomposed into a mechanical part, \( F_e \), and a swelling part, \( F_s \):

\[
F = F_e F_s, \quad (1)
\]

where \( F_s = \varphi^{-1/3} I \) and \( \varphi \) is the polymer network volume fraction of the hydrogel. The polymer network volume fraction is expressed as \( \varphi = 1/(1 + vc) \), where \( v \) is the volume per solvent molecule and \( c \) is the number of solvent molecules per polymer network volume. Since the gel is initially swollen, we define the swollen undeformed configuration as the reference configuration, and a deformation gradient \( f \) mapping from the stress-free reference configuration to the final swollen deformed configuration:

\[
f = \varphi_0^{1/3} F, \quad (2)
\]

where \( \varphi_0 \) is the polymer network volume fraction in the reference state. The left Cauchy-Green deformation tensor and its first invariant are defined as, \( b = F F^T \) and \( I_b = \text{tr}(b) \). The \( b \) tensor can be expressed in terms of its principle values and principle directions as:

\[
b = \sum_{i=1}^{3} \lambda_i^2 n_i \otimes n_i, \quad (3)
\]

and \( \lambda_i = \varphi_0^{1/3} \lambda_i \) are the corresponding principal stretches of \( f \) tensor. The change of volume from the initial dry configuration to the final configuration is related to the mechanical component and swelling component as: \( J = \text{det}[F] = \text{det}[F_e] \text{det}[F_s] = I_e \varphi^{-1} \).

We assumed that the free energy density of the hydrogel could be additively decomposed into a mechanical term arising from the stretching of a polymer network and a term describing the mixing energy of the polymer network and solvent system:
\[ \Psi = \Psi_{\text{mechanical}}(I_b, J_e) + \Psi_{\text{mixing}}(\varphi), \] (4)

The quasi-incompressible Neo-Hookean model is used to describe the strain energy of the network (39):

\[ \Psi_{\text{mechanical}}(I_b, J_e) = \frac{G}{2} (I_b - 3 - 2 \log J) + \frac{K}{4} (J_e^2 - 2 \log J_e - 1), \] (5)

where \( G \) and \( K \) are the shear modulus and bulk modulus of the polymer network respectively.

The Flory-Huggins model (23) is used to express the free energy of mixing:

\[ \Psi_{\text{mixing}}(\varphi) = \frac{RT}{v \varphi} [(1 - \varphi) \log(1 - \varphi) + \chi \varphi (1 - \varphi)], \] (6)

where \( R \) is the gas constant, and \( \chi \) is the Flory-Huggins parameter.

The Cauchy stress tensor is derived from the free energy density as

\[ \sigma = \sum_{i=1}^{3} \left\{ \frac{G q_0}{\lambda_i \lambda_2 \lambda_3} (q_0^{2/3} \lambda_i^2 - 1) + \frac{K q_0}{2 \lambda_i \lambda_2 \lambda_3} \left[ \left( \frac{q_0}{\lambda_0 \lambda_2 \lambda_3} \right)^2 - 1 \right] \right\} n_i \otimes n_i, \] (7)

\[ \mu = RT \left[ \log(1 - \varphi) + \varphi + \chi \varphi^2 \right] - \frac{K v \varphi}{2} \left[ \left( \frac{q_0}{\lambda_0 \lambda_2 \lambda_3} \right)^2 - 1 \right]. \] (8)

The constitutive model was implemented into TAHOE (Sandia National Laboratories) for finite element simulation of hydrogel structures. The shear modulus of the DNA hydrogel was obtained from swelling experiments and finite element analysis of the folding of BIS/DNA hydrogel bilayer beams. For the swelling experiments of BIS/DNA hydrogel bilayer beams, the dimensions in the hydrated state before adding DNA hairpins were \( l = 4.925 \) mm in contour length and \( w = 0.528 \) mm in width. The thicknesses of the DNA and BIS layers were \( t_{\text{DNA}} = 60.6 \) \( \mu \)m, \( t_{\text{BIS}} = 71.6 \) \( \mu \)m. The initial bilayer curvature in the hydrated state was measured to be 0.2 mm\(^{-1}\). DNA hairpins were added to the bilayer bars to induce sequence-driven swelling. The average curvature of swollen bilayer bars under equilibrium was measured to be 1.0 mm\(^{-1}\). The dimensions of both layers in equilibrium were also measured and the volumetric swelling ratios of the DNA gel and BIS gel were calculated to be 6.91 and 4.42 respectively. The sequence-induced swelling of the BIS gel was caused by the interpenetration of DNA and BIS gels during the layer-by-layer fabrication process, which was confirmed by confocal imaging.

Finite element model of swelling-induced folding of a hydrogel bilayer

For the finite element model of the bilayer beam, the simulation started from the initial hydrated configuration, where the initial curvature of bilayer was 0.2 mm\(^{-1}\) as measured in the
experiments. The model geometry had the same dimensions as measured for the fully hydrated photopatterned bilayers (Fig. S19). The mesh was discretized using trilinear hexahedral elements. The dimensions of the elements were \( l_e = 61.6248 \, \mu \text{m} \) in length and \( w_e = 52.8 \, \mu \text{m} \) in width, and the thicknesses were \( t_e^{\text{DNA}} = 15.15 \, \mu \text{m} \), \( t_e^{\text{BIS}} = 17.9 \, \mu \text{m} \) for DNA and BIS gel elements, respectively. The displacements at \( X=0 \), \( Y=0 \) and \( Z=0 \) were fixed. The Young’s modulus of the BIS gel in the fully hydrated state was measured to be 2.2 kPa from unconfined compression tests (Fig. S15). Assuming that the gels were mechanically incompressible resulted in 733 Pa for the shear modulus of the BIS gel. The bulk modulus was set to \( K = 1000G \) to enforce mechanical incompressibility of the polymer network. Though the BIS gel exhibited DNA interpenetration, we assumed that the shear modulus of the BIS gel did not change significantly during DNA-driven swelling. It was difficult and expensive to prepare and fully expand a DNA-crosslinked hydrogel via hairpin incorporation (in a 2% terminating hairpin solution) large enough to perform a compression test using our equipment because of the time required for diffusion of enough DNA hairpins into a very thick gel. Handling expanded hydrogels was also very difficult due to the softening that occurs during DNA-driven swelling. To determine the modulus of DNA gel in the fully swollen state, we therefore applied the finite element model to determine the shear modulus of the DNA gel needed to obtain the curvature measured for the actuated bilayer. The DNA sequence-driven swelling was simulated by varying the Flory-Huggins interaction parameter \( \chi \). The Flory-Huggins interaction parameter for each domain in the hydrated and DNA-actuated states were selected so that the free swelling ratio of the gels in the simulation matched the results from swelling experiments of BIS/DNA hydrogel bilayers (Fig. S14). The average volumetric swelling ratios measured for 12 specimens were 6.91 and 4.42 for the DNA and BIS layers, respectively. The parameters used in the model are listed in Table S2.

The initial polymer network volume fraction \( \varphi_0 \) was obtained by solving equations (7) and (8) with the conditions \( \sigma = 0 \) and \( \mu = 0 \). For the BIS and DNA gel domains, the Flory-Huggins interaction parameter was continuously decreased from 0.55 to 0.51, and at each value the deformation gradient field \( f(X) \) and polymer network volume fraction \( \varphi(X) \) were determined by finite element analysis. We performed the finite element simulations while varying the shear modulus of the DNA gel from 2.29 to 350 Pa, and calculated for each case the equilibrium curvature of the bilayer bar at the equilibrium swelling. The equilibrium curvature of the bilayer was calculated as follows. The deformed positions of the points on the midline of the bilayer inner surface were obtained from the simulation result. We determined the radius of the best fit circle to the points using the method of least squares. The curvature was then calculated by taking the inverse of the radius. The calculated curvature of the bilayer was compared to the curvature of the actuated bilayer measured in experiments to determine the equilibrium shear modulus of the DNA gel. A shear modulus of 229 Pa produced the best fit to the experimentally measured bilayer average curvature at steady state.

Parameter Study

We applied the model to investigate the effect of the thickness, shear modulus and volumetric swelling ratio of the DNA gel on the curvature of folding BIS/DNA gel bilayer bars. We first varied the DNA gel thickness from 13 \( \mu \text{m} \) to 70 \( \mu \text{m} \) while keeping the BIS layer thickness of
71.6 µm, DNA gel shear modulus of 229 Pa and volumetric swelling ratio of 6.91 unchanged. Next, we kept the DNA gel thickness and volumetric swelling ratio constant at 60.6 µm and 6.91, respectively, and adjusted the DNA gel shear modulus from 2.29 to 350 Pa. We then kept the DNA gel thickness of 60.6 µm and shear modulus of 229 Pa unchanged, and varied the DNA gel volumetric swelling ratio from 5 to 12. These parameters were varied independently and in each simulation the average equilibrium curvature of actuated BIS/DNA gel bilayer was calculated using the method described in the above section.

Theoretical solutions for the curving of thin film-thick substrate bilayer system have been derived based on the century-old Stoney formula (40) for stresses in the deposited thin film. This formula assumes small strains and rotations, and that the material of each layer is isotropic, homogeneous, and linear elastic. For the case where the thicknesses of each layer are comparable (e.g., \( t_{\text{BIS}} \sim t_{\text{DNA}} \)), Freund et al. (24) derived a modified Stoney formula for the bilayer curvature, which for an initially flat bilayer can be expressed as, \( K = 2\eta \). The bilayer ratio, \( \eta \), is given by,

\[
\eta = \frac{E_{\text{DNA}} t_{\text{DNA}}}{E_{\text{BIS}} t_{\text{BIS}}} \Delta \theta (1 + \frac{t_{\text{DNA}}}{t_{\text{BIS}}})
\]

for the case when the Poisson's ratio is the same in each layer. The \( E_{\text{DNA}} \) and \( E_{\text{BIS}} \) are the Young's moduli of the DNA and BIS gels (Pa); \( t_{\text{DNA}} \) and \( t_{\text{BIS}} \) are the thicknesses of DNA and BIS gel layers (mm); and \( \Delta \theta \) is the difference in the volumetric swelling ratio between the DNA and BIS gels. The modified Stoney formula provided a poor prediction of the simulation results for the curvature of the BIS/DNA gel bilayer, likely because the simulations exhibited large deformation and nonlinear elastic behavior, which violated the assumptions of the theory. However, we found that the simulation results for the curvature change scaled with the bilayer ratio. Plots of the curvature as a function of the bilayer ratio for all cases of the parameter study fell on a straight line of the form \( K = C\eta + K_0 \) (Fig 3B in the main text). A linear regression returned \( C = 0.21 \) for the proportionality constant and \( K_0 = 0.2 \text{ mm}^{-1} \) for the initial curvature, which agreed with the average initial curvature of the bilayers measured in the hydrated state \( 0.2057 \text{ mm}^{-1} \).

The bilayer ratio \( \eta \) depends nonlinearly on the modulus and thickness ratio of the DNA and BIS gels, and depends linearly on the difference in the volumetric swelling ratio. Supplemental Figure S20 shows the variation of the equilibrium bilayer curvature with the DNA gel shear modulus, BIS gel shear modulus, DNA gel thickness and volumetric swelling ratio. Increasing the DNA gel thickness or shear modulus increased the flexural stiffness of the DNA gel resulting in a higher curvature, signifying a more curved bilayer. Likewise, increasing the DNA gel volumetric swelling ratio increased the curvature. As shown in the figure, the curvature was most sensitive to the DNA gel volumetric swelling ratio. Changing the BIS gel shear modulus had a small effect on the final curvature, with a less stiff BIS gel leading to only slightly more folding. In contrast, the shear modulus of the DNA gel had a pronounced effect. When the DNA gel shear modulus was increased from 2.29 to 850 Pa, the equilibrium curvature increased asymptotically until the DNA gel shear modulus reached the BIS gel shear modulus. In Figure 3C of the main text we plotted the equilibrium bilayer curvature as a function of the DNA layer thickness \( t_{\text{DNA}} \) for BIS layer thicknesses of 51.6 µm, 71.6 µm, and 91.6 µm. Increasing the BIS gel thickness...
resulted in a lower curvature due to the increased flexural stiffness of BIS layer. However, the effect of varying the thickness of the DNA gel was more complicated. For each BIS gel thickness, there was an optimum thickness of DNA gel for which the bilayer curvature was maximized. For example, the optimal DNA gel thickness was 60.6 µm for a BIS gel thickness of 71.6 µm. The bilayer curvature decreased for larger and smaller values of DNA gel thickness. A DNA gel layer that is too thin did not exert enough force to bend the bilayer, while a DNA gel layer that is too thick was negligibly affected by the BIS gel layer and underwent uniform swelling rather than inducing folding. The optimal thickness increased with the BIS gel thickness. For the BIS gel thicknesses of 51.6 µm, 71.6 µm and 91.6 µm, the optimum DNA gel thicknesses were 42, 60.6 and 69.8 µm, respectively.

The parameter study also showed that the high degree of swelling of the DNA gel should allow millimeter to centimeter sized bilayer structures to achieve a large shape change. For example, we asked whether a 10 mm long flat bilayer beam that was also several millimeters thick (as opposed to 0.1 mm or less as we had studied previously) could fold into a complete circle. The relation obtained from the parameter study was $K = 0.21\eta + 0.2$, where 0.2 mm$^{-1}$ represents the initial curvature of the bilayers in the hydrated state. Using the relation $K = 0.21\eta$ and assuming the 10 mm long bilayer beam was initially flat, we determined that the 10 mm long bilayer with the optimum DNA gel thickness can be as thick as 7.23 mm and still fold into a complete circle for the maximum experimentally measured swelling ratio of 3.72 ± 0.11.
Fig. S1. Chemistry for synthesizing a poly(Am-co-BIS) or poly(Am-co-DNA) hydrogel. Both poly(Am-co-BIS) and the poly(Am-co-DNA) hydrogels were prepared by standard UV-initiated, radical copolymerization chemistry. In the case of poly(Am-co-DNA) gels, a pre-annealed DNA duplex—with each strand modified at the 5' end with a standard, commercially available, acrydite moiety—was used as a crosslinker. The acrydite-modified DNA strands were obtained from IDT in their lyophilized form.
Fig. S2. Hydrogel expansion driven by a single- to double-stranded crosslink transition. (a) To quantify the amount of hydrogel expansion that would result from a simple hybridization process within hydrogel crosslinks, we use a 3-strand crosslink architecture studied previously by Lin et al (11). Hydrogel squares with these crosslinks were fabricated according to the protocol listed in Methods (22). The resulting structures swelled to equilibrium due to solvent uptake in TAE/Mg$^{2+}$ buffer, after which the samples were immersed in TAE/Mg$^{2+}$ buffer containing 33.3 µM of F1 DNA strand that is complementary to the single stranded region within the crosslink. (b) Representative images of samples before and after treatment with F1. Approximately 24 hours after the addition of F1 strand, the gels had swelled uniaxially by roughly 5%. Brightness and contrast of the images was adjusted using ImageJ. Scale bars are 2 mm.
To determine the stiffness of the DNA-crosslinked hydrogels used in this study, elastic moduli measurements were obtained for a fully hydrated poly(Am-co-DNA) hydrogel. (a) A PDMS micromold was prepared by mixing base and curing components of Sylgard 184 in a 1:10 volume ratio. The resulting solution was poured over a negative pattern, wooden cubes, in a petri dish. The mixture was then heated at 70 °C for one hour and allowed to cool to room temperature. The PDMS mold was then peeled off the negative pattern and taken out of the petri dish. A poly(Am-co-DNA) gel cube sample with dimensions of roughly 4.7 x 4.7 x 4.7 mm was prepared by photopolymerization of a system 1 pregel solution in the PDMS mold. The gel was exposed to 365 nm UV light with an intensity of 7.55 mW/cm$^2$ for five minutes to ensure complete curing of the pregel solution. The gel sample was then placed in 3 mL of fresh TAE/Mg$^{2+}$ buffer and was allowed to swell due to solvent uptake to equilibrium over a period of roughly two weeks, with the buffer being replaced approximately every 3 days. (b) The elastic modulus of the DNA gel sample was measured using a controlled force, unconfined compression test at room temperature (Q800 DMA; TA instruments). The applied force on the gel sample was ramped to a maximum static force of 2 mN at a rate of 1 mN/min. Once the maximum static force was reached, the applied force was ramped down to 0 N at the same rate. Static force and displacement data for the loading portion of the compression test were used to generate true stress and true strain curves for the sample. The elastic modulus was measured as the slope of the best-fit line to the stress-strain curve. Previously D. C. Lin et al. reported that the elastic moduli for a poly(Am-co-DNA) gel ranges from 59 Pa to 11.6 KPa depending on crosslink density (41), consistent with these measurements.
Fig. S4. Long-term stability of photopatterned poly(Am-co-DNA) gel architectures. Poly(Am-co-BIS)/poly(Am-co-DNA) crab architectures were prepared as described in Methods and Fig. S17. The micrographs show a typical crab (left) after fabrication and immersion in TAE/Mg$^{2+}$ buffer for 24 hours, and then (right) the same structure after storage at 4 °C in TAE/Mg$^{2+}$ buffer for roughly five months. Prior to imaging the crab after storage, the sample was flipped over in the course of handling. Scale bar is 2 mm.
Fig. S5. The degree of swelling of poly(Am-co-DNA) gels can be controlled by adjusting the relative percentages of terminator and polymerizing hairpins. To assess the degree of expansion, poly(Am-co-DNA) gel squares and hairpin solutions with a total hairpin concentration of 20 µM of each of the two hairpin types (e.g. H1, H2) – with the percentages of terminator shown in the legend – were prepared following the protocols listed in the Methods section. Before the gel squares were added to the hairpin solution, they were allowed to take up buffer in a DNA-free solution for 24 hours. This DNA-free solution also contained 2x SYBR Green I nucleic acid stain to enable the gels to be imaged via fluorescence during swelling. For each percentage listed, 4 hydrogel squares were mixed with 3 mL of buffer containing the corresponding hairpin concentrations in a standard petri dish. After the gels were added to the hairpin solutions, images of the gels were captured every 20 minutes in standard gel imager. At each time point, all four sides of each DNA gel sample were measured manually and averaged, then divided by the average lengths of the sides at time zero to obtain a uniaxial swelling measurement, which we denote as $\Delta L/L_0$. For samples that curled during expansion (some of the 0% and 2% terminator samples), the lengths of observable sides were averaged to calculate the degree of uniaxial swelling. Samples were tracked for 36 hours. Data for the 0% sample is not shown after 24 hours because the squares dimmed and their size could no longer be tracked reliably. Error bars represent a single standard deviation about the mean swelling value (N = 4).
Fig. S6. Swelling of poly(Am-co-DNA) films of different thicknesses. The swelling kinetics of 1x1 mm, system 1 poly(Am-co-DNA) hydrogel squares, with measured thicknesses of 44 µm ± 3 µm and 136 µm ± 2 µm (mean ± SD), in response to system 1 hairpins. The thickness was measured before the addition of hairpins for 4 samples using a confocal microscope as described in Methods. Uniaxial swelling is averaged for at least three samples for each thickness. Error bars represent a single standard deviation about the mean swelling value.
Fig. S7. Swelling of poly(Am-co-DNA) films with different hairpin toehold lengths. (A) DNA crosslinker and hairpin systems containing either 3, 4 or 6 base pair toeholds were designed. The altered domains are indicated with arrows. The DNA crosslinker and hairpin system with the 3 bp toeholds are designated in the main text as “system 1.” (B) The crosslinker complexes were prepared by annealing strand C with a C’ strand containing 3 bp, 4 bp, or 6 bp toehold regions according to the protocol outlined in the Methods section. The 1 mm x 1 mm x 60 µm photopatterned poly(Am-co-DNA) hydrogel squares were swelled via the addition of a 20 µM, 2% terminator hairpin solution consisting of polymerizing and terminator hairpins with regions complementary to the 3 bp, 4 bp, or 6 bp toehold regions. Uniaxial swelling values are averaged for at least three samples for each toehold length. Error bars represent a single standard deviation about the mean swelling value.
Fig. S8. Swelling of poly(Am-co-DNA) films with different total hairpin concentrations. The swelling kinetics of 1 mm x 1 mm x 14 µm photopatterned poly(Am-co-DNA) hydrogel squares containing system 1 crosslinker. The samples were placed in 3 mL of buffered solution containing 10, 20, 30 or 40 µM of overall hairpins, 2% of which was terminator hairpin monomers. Uniaxial swelling values are averaged for at least 3 samples for each hairpin concentration. Error bars represent a single standard deviation about the mean swelling value.
Fig. S9. DNA-driven expansion of poly(Am-co-DNA) gels crosslinked by different sequences in response to their respective polymerizing hairpins. Poly(Am-co-DNA) gel samples were prepared as described in the Methods section with either system 2, system 3 or system 4 crosslink complexes. All gel samples were 1 mm x 1 mm x 60 µm in size. To visualize the gels, samples were stained overnight in 2x SYBR Green I nucleic acid stain and subsequently washed in fresh TAE/Mg^{2+} buffer before being added to a 20 µM hairpin solution containing either 0% or 2% terminator hairpin and were monitored via fluorescence microscopy. Sample dimensions were measured manually using ImageJ software. The error bars show a single standard deviation about the mean swelling value of at least four samples in a particular hairpin solution.
Fig. S10. Poly(Am-co-DNA) gels do not expand in solutions of non-complementary hairpin types. Poly(Am-co-DNA) gels containing either system 1 or system 2 crosslinks were prepared following the protocol listed in the Methods section. The gels were stained overnight in 2x SYBR Green I nucleic acid stain and were subsequently washed in fresh TAE/Mg\(^{2+}\) buffer prior to adding the DNA hairpin solution. Four gel samples crosslinked with system 1 DNA complexes were placed in a 20 µM solution of system 2 polymerizing hairpins (with 0% terminator). Conversely, four gel samples crosslinked with system 2 DNA complexes were placed in a 20 µM solution of system 1 polymerizing hairpins (with 0% terminator). The gels were monitored via fluorescence microscopy; sample dimensions were manually measured using ImageJ software. The error bars represent a single standard deviation about the mean swelling value of all the samples exposed to a given hairpin solution.
Fig. S11. Sequence-specific incorporation of hairpins into poly(Am-co-DNA) gels during expansion. To verify that DNA hairpins accumulate in poly(Am-co-DNA) gels when expansion occurs but not otherwise, two sets of poly(Am-co-DNA-co-rhodamine) gels – one crosslinked with system 1 and the other crosslinked with system 2 – were each first exposed to a solution of system 1 hairpins, then to a solution of system 2 hairpins. The H1 polymerizing hairpin in each system was labeled on the 5’ end with a FAM fluorophore. The system 1 gel was patterned as a triangle, whereas the system 2 gel was patterned as a square so that the type of crosslinks within the gel could be identified by the gel’s shape. Before the addition of hairpins, both samples were visible under a Nikon AZ100 epifluorescence microscope using a G-2E/C filter cube (528-533 nm excitation, 590-650 bandpass) because of the rhodamine dye; no significant fluorescence was observed when imaging with the FAM filter. After the addition of 19.6 µM of FAM-labeled system 1 hairpins and 0.4 µM unlabeled system 1 terminator hairpins (i.e. a 2% fraction of the total hairpins, following other experiments), the gel with system 1 crosslinks (the triangle) expanded and was readily visible in the FAM channel, whereas the other shape was not visible. The samples were then transferred to a solution of 19.6 µM of FAM-labeled system 2 hairpins and 0.4 µM unlabeled system 2 terminator hairpins (i.e. a 2% fraction of the total hairpins, following other experiments). In this solution the system 1 crosslinked-gel did not change significantly in size or brightness, but the system 2-crosslinked gel expanded and became visible in the FAM channel. Scale bars are 1 mm.
Fig. S12. Process Diagram for poly(Am-co-BIS) / poly(Am-co-DNA) Hydrogel Bilayer Fabrication. Parameters such as bake temperature/time, spacer thickness and solution concentrations are given in the Methods section. After each UV exposure, the resulting samples were washed with TAE/Mg^{2+}/Ca^{2+} buffer to remove unpolymerized monomers and DNA crosslinks. The calcium cations in the buffer prevent degradation of the ionic crosslinks of the poly(acrylic acid) sacrificial layer.
Fig. S13. Swelling of poly(Am-co-BIS) and poly(Am-co-DNA) hydrogels after patterning due to solvent uptake. Both the poly(Am-co-BIS) and poly(Am-co-DNA) gels swell in TAE/Mg\(^{2+}\) buffer after photopatterning due to solvent uptake. This solvent uptake controls the initial size of the gels when DNA is added to induce specific actuation. Differential swelling of poly(Am-co-BIS) and poly(Am-co-DNA) gels due to solvent uptake caused the bilayers in Fig. 3 to curve slightly before DNA-driven actuation, as seen in Fig. 3A. To measure the extent of swelling through solvent uptake for each gel type, poly(Am-co-BIS) and poly(Am-co-DNA) hydrogels were photopatterned as described in Methods. The poly(Am-co-BIS) gels were labeled with rhodamine B via copolymerization with acrylamide and BIS, whereas the poly(Am-co-DNA) gels were stained overnight in 2x SYBR Green dye. The gels were then allowed to equilibrate in TAE/Mg\(^{2+}\) buffer for 24 hrs. The poly(Am-co-BIS) gels swelled uniaxially due to solvent uptake by an average of 0.12 ± 0.04 (mean ± SD, N = 5). The poly(Am-co-DNA) gels uniaxially swelled due to solvent uptake by an average of 0.36 ± 0.04 (mean ± SD, N = 4). Images of sample (a) poly(Am-co-BIS) and (b) poly(Am-co-DNA) gels after 24 hours of equilibration in solvent are shown. The degree of swelling was calculated using pattern dimensions of the photomask as the initial lengths of the gel edges. Scale bars are 1 mm.
Fig. S14. Kinetics of BIS/DNA bilayer actuation. Gel bilayer architectures comprised of a bottom poly(Am-co-BIS) layer and a top poly(Am-co-DNA) layer were fabricated as described in Methods and Fig. S12. After fabrication, the gel structures were allowed to equilibrate in TAE/Mg$^{2+}$ for at least 24 hours. Next, the samples were placed in 3 mL of 20 µM hairpin solution containing 2% terminator hairpin. The curvature of the samples was monitored via fluorescence microscopy and was measured using ImageJ software. Each of the data points represents measurements from 3 samples. Error bars represent a single standard deviation about the mean curvature value.
Measurement of the Young’s modulus for poly(Am-co-BIS) hydrogels. Three poly(Am-co-BIS) hydrogel samples, each 8 mm x 8 mm x 8 mm in size, were fabricated via photopolymerization of 5% Am:BIS (19:1) pregel solution in a previously prepared PDMS mold (see Fig. S3). The hydrogel samples were then placed in 1x TAE/Mg\(^{2+}\) for two days to swell to equilibrium via solvent uptake. To obtain elastic moduli values for the gels, the samples were subjected to an unconfined compression, controlled-force deformation test (Q800 DMA, TA instruments). The applied force was increased at a rate of 0.01 N/min until a maximum static force of 0.015 N was reached, after which the load was reduced at the same rate to 0 N. True stress-strain curves were generated from the raw static force and displacement data. Elastic moduli values were calculated as the tangent to the best fit quadratic curve at 1% strain, and were determined to be 2.24 kPa, 2.18 kPa and 2.17 kPa for samples 1, 2 and 3, respectively. They are on the order of previously determined elastic moduli values for poly(Am-co-BIS) gels prepared with similar concentrations of Am and BIS (42).
**Fig. S16. Flower fabrication.** Parameters such as bake temperature/time, spacer thickness and solution concentrations are listed in Methods.
**Fig. S17. Crab fabrication.** Parameters such as bake temperature/time, spacer thickness and solution concentrations are listed in Methods.
Fig. S18. BIS/DNA bilayer crab stability after actuation. Poly(Am-co-BIS)/poly(Am-co-DNA) crab architectures were prepared according to the protocol described in Methods and Fig. S17. Each DNA domain of the bilayers was actuated via treatment with a solution containing 20 µM of the systems 1-3 hairpins, with 2% terminator, for at least 24 hours at room temperature. The actuated crab bilayers then were stored in this same buffer at 4 °C for 2 months. The samples were imaged after the room temperature incubation (left) and after storage at 4º C for two months (right) via fluorescence microscopy. Scale bars are 2 mm.
Fig. S19. Finite element model of the BIS/DNA hydrogel bilayer. Green represents poly(Am-co-BIS) hydrogel and red represents poly(Am-co-DNA) hydrogel.
Fig. S20. Computational predictions of bilayer curvature as DNA gel swelling ratio, DNA gel thickness, and DNA and BIS gel shear moduli are individually varied. (A) DNA gel volumetric swelling ratio was changed from 5 to 12, while a DNA gel thickness of 60.6 µm, DNA gel shear modulus of 229 Pa, BIS gel thickness of 71.6 µm and BIS gel shear modulus of 733 Pa were kept constant; (B) DNA gel thickness was changed from 0.013 to 0.07 mm, while a DNA gel shear modulus of 229 Pa, DNA gel volumetric swelling ratio of 6.91, BIS gel thickness of 71.6 µm and BIS gel shear modulus of 733 Pa were kept constant; (C) DNA gel shear modulus was changed from 2.29 to 850 Pa for a BIS gel shear modulus of 633–833 Pa, while a DNA gel thickness of 60.6 µm, DNA gel volumetric swelling ratio of 6.91 and BIS gel thickness of 71.6 µm were kept constant; (D) BIS gel shear modulus was changed from 233 to 1433 Pa while a DNA gel shear modulus of 229 Pa, DNA gel thickness of 60.6 µm, DNA gel volumetric swelling ratio of 6.91 and BIS gel thickness of 71.6 µm were kept constant.
Table S1. DNA sequences of crosslinker and hairpin systems. All sequences were ordered from IDT in their lyophilized form and resuspended with TAE/Mg\(^{2+}\). Sequences for acrydite-modified strands are preceded with a /5ACryd/ designation.

### System 1 Strands

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Table S2: Parameters determined for DNA and BIS hydrogels.

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**Movie S1: DNA-hairpin driven expansion of a poly(Am-co-DNA) gel in a solution of 20 µM polymerizing hairpins.** Time lapse fluorescence video of a 1 mm x 1 mm x 60 µm, SYBR Green-stained poly(Am-co-DNA) gel over 44 hours. The video is shown 8,300x faster than real time. The gel sample was visualized using a Syngene gel imager and blue light transilluminator (see Methods). Individual frames were subjected to histogram clipping and contrast stretching in MATLAB.

**Movie S2: DNA-driven expansion of a poly(Am-co-DNA) gel in a solution of 98% polymerizing hairpin and 2% terminator hairpins.** Total hairpin concentration is 20 µM. Time lapse video of a SYBR Green-stained, 1 mm x 1 mm x 14 µm poly(Am-co-DNA) gel showing expansion to well-defined final size when exposed to a solution of polymerizing and terminator hairpin. The video is shown 8,600x faster than real time. The gel sample was visualized using a Syngene gel imager and blue light transilluminator (see Methods). Individual frames were subjected to histogram clipping and contrast stretching in MATLAB.
References and Notes


22. Materials and methods are available as supplementary materials.


