SPECIFIC DNA SEQUENCE RESPONISIVE DNA CROSSLINKED HYDROGEL AND ITS APPLICATIONS

by

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Abstract

In recent decades of years, stimuli-responsive hydrogel and microgel have been widely applied in a variety of research fields from drug release to shape-change architecture. The responsive capability to environment makes hydrogel attractive. Researchers have showed hydrogel can respond to several non-specific stimuli as changes in temperature and pH. But for some applications in which a steady-state environment is required, we need a specific signal which only actuate the hydrogel without changing the physical properties of environment. In this thesis, we report a DNA crosslinked hydrogel responsive to specific DNA sequence with massive volumetric swelling. By designing the DNA crosslinker, we can make a series of hydrogel responding to different DNA sequence. Because the DNA sequence can further be modified with other biomolecules or fluorescent probe, this technology is able to build a bridge between molecular level change and macroscopic change.

In the first theme of the thesis, we show hydrogel architectures photopatterned by DNA co-polymer that can be actuated by up to three different DNA sequence. The hydrogel architectures show great shape change accurately at the region we design after the specific biomolecular signal is applied to the system. This combination of microfabrication technology with DNA nanotechnology allow us to build tiny soft devices or soft robots that can be actuated by particular DNA sequence.

The second theme of this thesis is generating DNA crosslinked microgel through microfluidic methods. To apply the method, we first form emulsion droplets

out of microfluidic device then solidify the droplets by subsequent UV triggered polymerization. In this droplets generating process, by tuning the flow rate of the continuous phase and the dispersed phase, microfluidic generation of droplets can be well controlled over size and monodispersity. With this strategy, we can generate a great amount of microgels with uniform spherical geometry in a relatively simpler process and a faster speed than microfabrication and template particle synthesis.

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1. Introduction

1.1 Background and motivation

1.1.1 DNA Nanotechnology

DNA are well known as vital "molecules of life" for their important role in heredity and expression of living organism information. Beyond the biological functions, DNA has been applied as fundamental brick for nanomaterial engineering.[1-4] Researchers use DNA to build structure at nanoscale, which provides innovative applications for amplified sensing, nano-machine and nano-medicine and so on. [5-6]DNA nanotechnology has been making remarkable progress in the last decades. The four bases -- adenine (A), guanine (G), cytosine (C) and thymine (T) -- constitute the building blocks of the DNA biopolymer via hydrogen bonding interactions following the Watson-Crick complimentary of A-T and G-C. Comparing to other bio-material or nano-material, DNA has an unique advantage that the DNA molecules attach and detach with known sequence, which allow us to build microscale or nanoscale systems with rational specific sequence design.

The idea of DNA based structure as engineering material was first proposed by Nadrian Seeman. It is said Seeman was inspired by the M. C. Escher woodcut *Depth* to generate the idea that DNA can construct a three-dimensional lattice. Later in 1991, Seeman's lab published the synthesis of a cube made of DNA lattice.[7] Subsequent efforts made by several laboratories developed this original idea, researchers can

construct different topological structures like nanotubes or lattices with high-degree resolution. Researchers also used DNA to build nano-scale machines responsive to specific sequence or environmental input. Different DNA-machines like DNA tweezers and DNA walkers were constructed in the past decades. [8-9] At its starting stage, it has already showed great potential as molecular level mechanical devices with programmable reaction to environmental triggers.

Another milestone of DNA nanotechnology is the development of DNA origami technology demonstrated by Paul Rothemund. In the DNA origami method, a long "scaffold" DNA strand folds into various shape with the help of several short complimentary "staple" strands. From a single smile face to a map of an country, this DNA origami allow us to build complex and programmable shapes with great yield up to 80%.[4] The DNA origami technology developed so quickly in the last decade, from symmetrical structure to asymmetrical structure, from two-dimensional structure to three-dimensional structure. Many early-stage application has been reported for DNA origami technology. The most attracting application must be its potential in drug delivery for its function as the carrier for nanoscale particles. However, DNA origami still has some limitations in itself. The length of the scaffold strand limits the size of the origami structure to the order of 100 nm, which make it difficult to design more adaptable structures. Once we can overcome the limits of size control of DNA origami, it can really become a powerful technology to design whatever nanoscale structure we want.

1.1.2 DNA Hydrogel or DNA crosslinked hydrogel

Hydrogel is a macromolecular polymer gel network with a large fraction of aqueous solvent attaching to its framework. With highly absorbent, hydrogel is considered as a great functional material in many research field. For example, hydrogel can be used as scaffold of tissue engineering to mimic human body microenvironment.[10-13] Also, "smart" gel can be used as sensor to environment changes like pH or temperature, furthermore people can use this sensing ability to build environment responsive drug delivery system. Hydrogel is considered as a great shape-change material for small scale soft robot, usually combined with 3D printing or photo lithography fabrication.[14]

DNA, as a natural biological polymer with remarkable biocompatibility, has been used to build hydrogel. In 2004, Langrana use DNA strand as crosslinker for polyacrylamide hydrogel. In that case, DNA strands modified with phosphoramidite group (trade name as Acrydite). [15]This modification link DNA strand with a Carbon-Carbon double bond to be polymerized with other monomers. Later, researchers also developed hydrogel entirely made of DNA. [16-17]As a biopolymer to store genetic information in living organism, DNA provide great control ability on scaffold pore size and potentially allow us to interact with hydrogel with DNA sequence information.

1.2 Thesis Overview

In this thesis, our goal is to report a new kind of DNA crosslinked hydrogel swelled with specific DNA input, and the application of the hydrogel. *Chapter 2* of the thesis talks about the mechanism of the hydrogel, the process of the hydrogel swelling and its application as soft-material, shape-change device. *Chapter 3* contains additional information and supporting information for *Chapter 2*. In *Chapter 4*, we describe a method to make DNA crosslinked microgel by microfluidic droplet formation. Chapter 5 contains additional information and supporting information for Chapter 4.

1.3 Contribution

A version of *Chapter 2* and *Chapter 3* has been submitted to publication. Angelo Cangialosi and Chang Kyu Yoon are the co-first-authors of the paper. Jiayu Liu, Jingkai Guo, myself, Thao Nguyen, David Gracias and Rebecca Schulman are coauthors of the paper. Angelo Cangialosi, ChangKyu and myself conducted the experiments. Jiayu Liu, Jingkai Guo and Thao Nguyen designed and conducted the simulation. All the authors discussed the result and wrote the manuscript.

2. DNA Crosslinked Hydrogel Architecture Shape-Change Driven By DNA Sequence

2.1 Introduction

Traditionally, robots are made from rigid materials like metal or plastic to make it stable and reliable. However, as we can see on live organism, structures are always soft and deformable. Inspired by the natural system, scientist have tried to develop robots with soft materials, refers as soft robots. Hydrogel as remarkable soft material, therefore considered as a good choice to make soft robot. There are several ways to pattern hydrogel into a shape, like molding, photo-lithography and 3D printing. In our case, we choose photo-lithography fabrication to make hydrogel architecture.

Non-specific signals have been used to trigger the shape-change of hydrogel like pH and temperature.[14, 18-20] In our research, we report how specific sequence DNA hairpin trigger the swelling up to 80-fold volumetric expansion of DNA crosslinked poly-acrylamide hydrogel. Furthermore, because of the unique codability of DNA, we can build several systems respond to different DNA sequences and independent to each other. The codability of DNA allow us build complex hydrogel architecture with programmable shape-change ability.

In the project, we also characterize the mechanical properties of the DNA crosslinked hydrogel and its shape change behavior using experiments and finite element simulations. Since DNA molecules can be coupled to molecular sensors,

amplifiers and logic circuits, these shape change devices introduce the possibility of building soft devices that respond to diverse chemical inputs and autonomously implement adaptive chemical control programs.

2.2 Experimental Design

We focused on hydrogels, where structural changes within crosslinks can drive expansion or contraction in aqueous media. We used DNA molecules because their hybridization is predictable and highly sequence-specific and because DNA hybridization can direct shape change of nanostructures, thin films and colloidal crystals. We considered DNA-crosslinked polyacrylamide hydrogels in which DNA duplexes crosslink polyacrylamide chains into a hydrogel network. We postulated that a DNA hybridization cascade in which DNA hairpins are inserted into crosslinks via strand displacement would trigger a high-degree of swelling. In a different context, this cascade has been previously shown to push nanoscale objects away from one another. To test this theory, we designed DNA sequences, which we denoted as system 1, for the hydrogel crosslinks and corresponding hairpins, (called H1 and H2) for the cascade.

Next, we developed a method to pattern the hydrogels into precisely defined shapes and architectures using photolithography. While numerous photolithographic processes for silicon-based devices exist, protocols for photopatterning DNA hydrogels are largely absent, and the patterning process presents unique challenges. Firstly, we started with photopatterned single layer. We tried a series of experiments

to actuate the swelling of hydrogel. We reported tremendous swelling actuated by corresponding hairpin. We also designed different systems and tested their swelling independence to each other. We next asked whether we can control the designed final size of hydrogel. We modified the sequences of the polymerizing hairpins H1 and H2 to create "terminator hairpins" that could binding into a crosslink but stop further DNA polymerization, which helped us get a controlled final swelling ratio of the hydrogel.

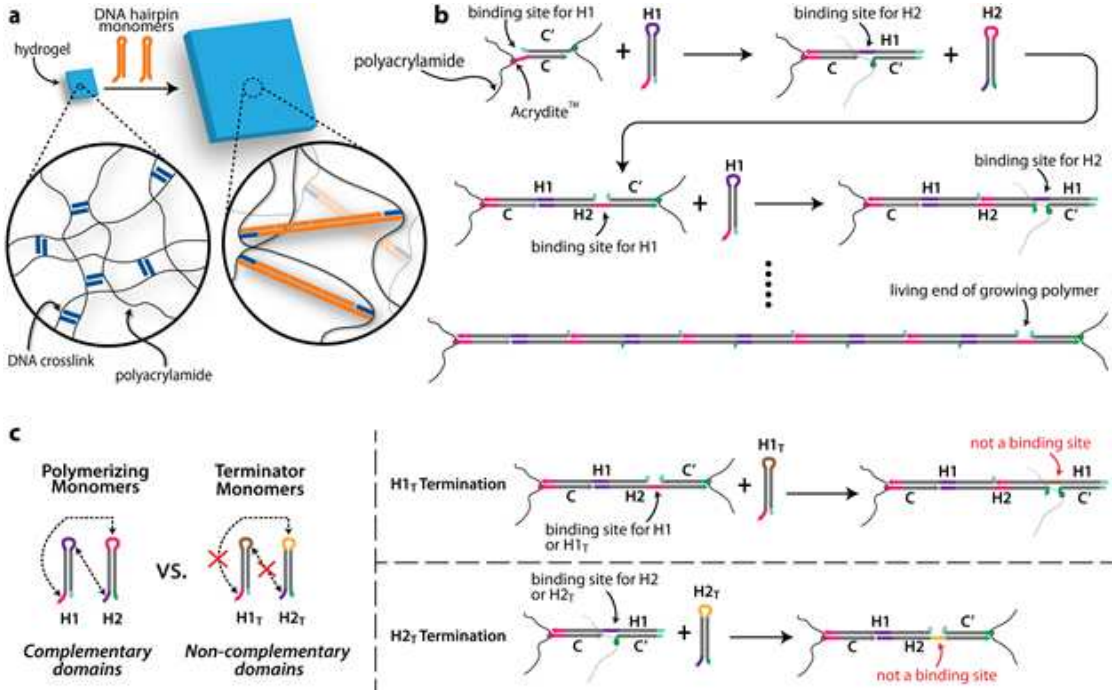

Figure 1. Expansion of DNA-crosslinked polyacrylamide gels through DNA hybridization-driven polymerization. (**a**) Polyacrylamide and hybridized DNA strands crosslinks form a hydrogel. Hairpins can insert into crosslinks, lengthening them and inducing hydrogel expansion. Polyacrylamide is shown in gray, DNA crosslinks in blue and inserted hairpins in orange. (**b**) Reaction schematic for the crosslink extension. C and C' are crosslinks, H1 and H2 hairpin monomers. Colors within DNA strands indicate sequence domain type; the same color is used for a domain and its complement. Thin gray lines are polyacrylamide. (**c**) Polymerizing hairpin monomers can insert into crosslinks and allow the insertion of additional monomers, while terminator hairpin monomers insert and leave a single stranded site that no monomers can interact with, thereby terminating polymerization. Color coding and notation of the reaction scheme are shown as in (**b**).

After we got a good understanding of monolayer hydrogel, we developed to build beam-shaped bilayer structure by fabricating a DNA-crosslinked hydrogel layer on the top of a BIS-crosslinked hydrogel layer. As we expected, the bi-layer curled a lot with the present of corresponding hairpins.

We applied finite element analysis to study bilayer folding caused by DNA-induced swelling. For the analysis, the bilayer structure was divided into discrete elements and the displacements at the element nodes were determined by solving the linear momentum balance for given boundary conditions and constitutive relations for the stress-strain-swelling behavior of the DNA- and BIS-crosslinked gels. Solvent diffusion occurs much more quickly through the micron-thick layers than the time-scale of folding and thus was neglected in the analysis. This allows us to apply a hyperelastic constitutive model that we developed previously to understand chemomechanical shape change to describe the coupled DNA-driven swelling and stress response of the bilayers. Briefly, the stress response of the gel is assumed to be the sum of an elastic component for the entropic response of the polymer network, and the solvent pressure acting on the network. We used Flory-Huggins theory to describe the chemical potential of the water in the network that gives rise to the solvent pressure. [21-22] We determined the final shape of the structure after DNA-driven swelling by changing the Flory-Huggins parameter in the DNA- and BIS-crosslinked gel layers to achieve the experimentally measured volumetric swelling ratios and then solving for the displacement field in the bilayer. We used the model to

investigate how DNA gel thickness, modulus, and the degree of expansion induced by DNA-driven swelling would be expected to control the final radius of curvature of DNA- and BIS-crosslinked gel bilayers.

We next explored how structures with multiple, different DNA sequence-responsive hydrogels would change shape differentially in response to different hairpin inputs. Hydrogel 6-petal flowers and crab devices were made combined systems of DNA crosslinked hydrogel. We actuated different parts of these devices with different systems of hairpins.

2.3 Preparation of pre-gel solution

For the DNA pregel solution, stock solutions of the crosslink strands A and R (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/Mg2+. Oligonucleotide concentrations were verified by absorbance spectroscopy at 260 nm. To prepare the DNA copolymer gel, referred to as poly(DNA-co-Am), the crosslink strands were added TAE/Mg2+ buffer supplemented with calcium chloride (Sigma #C1016) – herein referred to as TAE/Mg2+/Ca2+ – and MilliQ water. The calcium chloride was added to prevent premature dissolution of the polyacrylic acid (PAA) sacrificial layer. This solution

was 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 O2 on radical chain polymerization. The final concentrations of all pregel components are as follows: 1.154 mM of strands A and R, 1x TAE/Mg2+ 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/Mg2+/Ca2+ 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/Mg2+ buffer, and calcium chloride are the same as in the poly(DNA-co-Am) pregel solution. The final concentrations of the other species is 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.

2.4 Photolithography Process

The photolithography chambers were prepared according to a previously published protocol. 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 ºC 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 Nitrogen 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 Nitrogen 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 bottom slides of the photolithography chamber (Catalog #16004-424, VWR) were prepared by sonicating them in 10% (w/w) NaOH for 30 minutes, rinsing them with MilliQ water, and drying under Nitrogen gas. The bottom slide was then treated

with Oxygen 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) polyacrylic acid (PAA) crosslinked with calcium was then deposited onto the substrate according to a previously reported protocol. Additional washing (3 min in DI water) and baking steps (5 min at 150ºC) were added to the protocol to remove calcium salt deposits present on the substrate after crosslinking the PAA in a solution of $CaCl_2$. 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 pre-gel solution.

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/cm^2 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 1M 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.

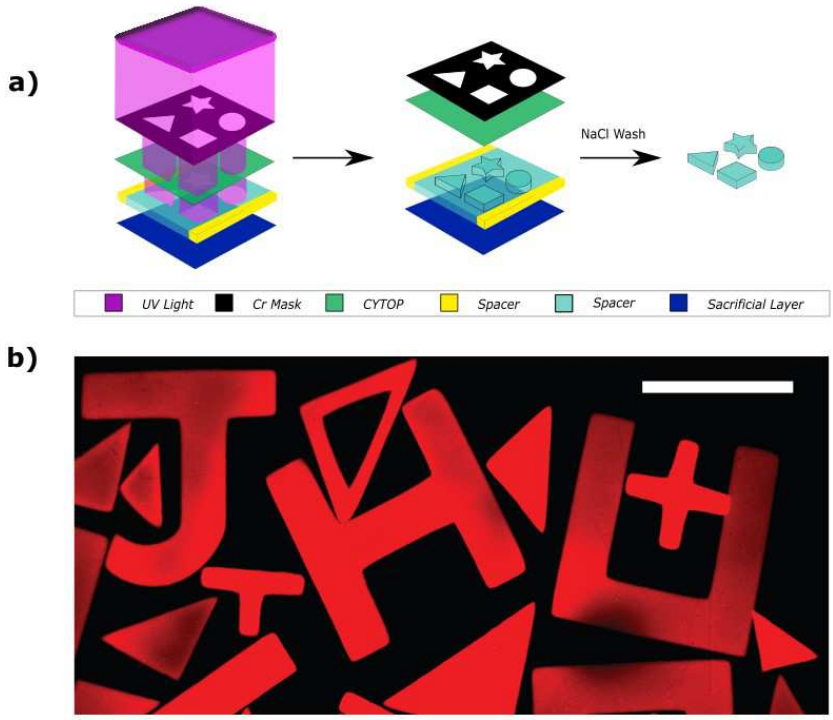

Figure 2 | Photopatterning and controlled expansion of DNA-crosslinked polymer hydrogels. (a) Photopatterning process developed for producing large numbers of hydrogel shapes and architectures. (**b**) Fluorescence micrograph of hydrogel shapes post-stained with Sybr Green I (False colored with MATLAB). Scale bar 5 mm.

To prepare the first hydrogel layer of a bilayer structure, the previous photopatterning protocol was followed using BIS-co-Am-co-fluorescein pregel solution except that after UV exposure the substrate was washed with approximately 200 µL of TAE/Mg2+/Ca2+ to remove unreacted pre-gel solution from the patterned structures. The UV dose for all BIS-crosslinked hydrogel structures is approximately 280 mJ/cm^2. 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 DNA-co-Am-co-rhodamine pre-gel solution. Prior to photopatterning, another 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 DNA-co-Am-co-rhodamine solution was injected via pipet into the photolithography chamber and exposed to UV light for a total dose of 240 mJ/cm^2. At this point, when the bilayer bar structures were fabricated, the chamber was gently disassembled and approximately 1 mL of 1M NaCl was aliquoted onto the substrate to dissolve the sacrificial layer and yield freestanding bilayer bar structures. When the petal or crab bilayer structures were fabricated, the above process of washing the patterned structures, aligning the masks, injecting pre-gel solution, and exposing to UV light is repeated until the final hydrogel domain is patterned, at which point the photolithography chamber is disassembled and 1M NaCl was added to yield free-floating hydrogel structures.

2.5 The Swelling Kinetics of Monolayer Hydrogel

Followed the protocol we demonstrated above, we fabricated 0.06 x 1 x 1 mm hydrogel squares with system 1 crosslinkers inside. We let them sit in 1x TAE/Mg2+ buffer for fully solvent uptake. Then we swelled the squares with corresponding hairpins, and we observed for system 1 DNA-crosslinked hydrogel expanded over 200 percent uniaxially, while the squares in buffer with alternate hairpins did not expand. We occasionally observed transient curling and uncurling during expansion, which may due to the incomplete polymerization of the hydrogel. If the expanding gels remained indefinitely in buffer containing H1 and H2, their expansion continued unabated until the gels were no longer visible.

In the next step, we wonder if we can get a desired final size of the hydrogel after swelling. We modified the sequences of the polymerizing hairpins H1 and H2 to create "terminator hairpins" that could insert into a crosslink but prevent subsequent insertions. Gels exposed to a mixture of these hairpin types swelled without curling to a well-defined final size that was controlled by the relative concentrations of polymerizing and terminator hairpins. Inclusion of 2% terminator hairpins (relative to polymerize hairpins) produced high-degree but well-controlled expansion and was used in the remainder of our studies.

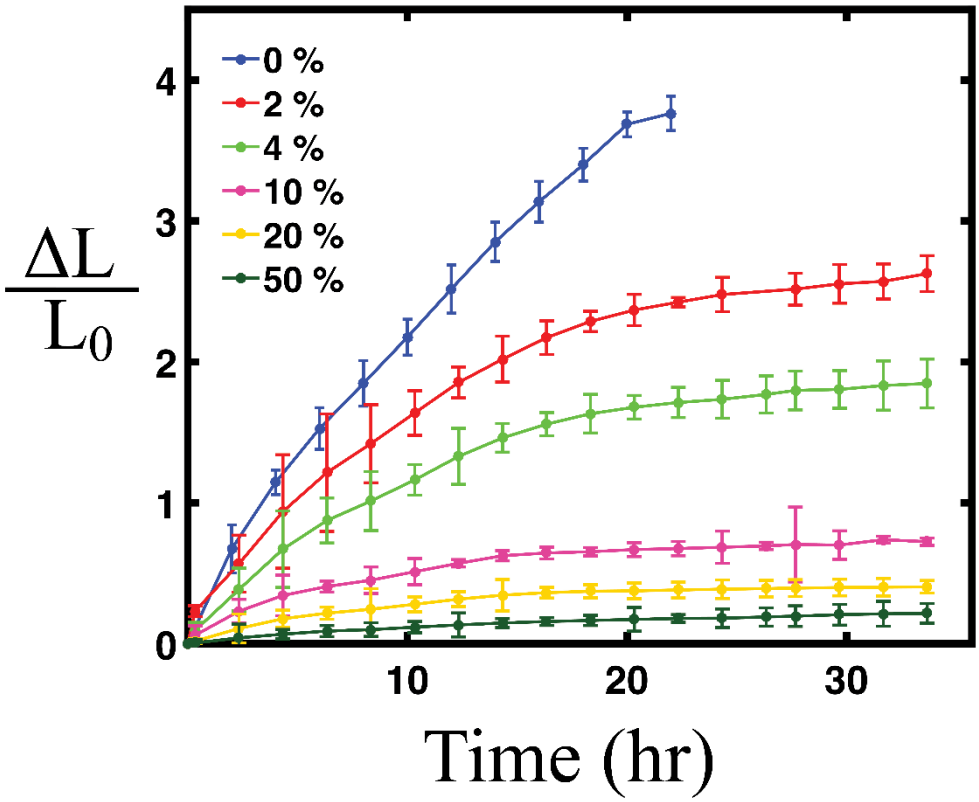

Figure 3 |The degree of swelling of poly(Am-co-DNA) gels can be controlled by adjusting the relative percentages of terminator and polymerizing hairpins. To assess the degree of expansion, poly(Am-co-DNA) gel squares and hairpin solutions to a total concentration of 20 μM with the percentages of terminator shown in the legend were prepared following the protocols listed in the Methods section and allowed to take up buffer in a DNA-free solution for 24 hours before further

processing. This DNA-free solution contained 2x SYBR Green I nucleic acid stain to enable gel tracking 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. All four sides of each DNA gel sample were measured manually and averaged to obtain a uniaxial swelling measurement, which we denote as $\Delta L/L_0$. Data points are the average of the uniaxial swelling values from at least three DNA gel samples in a given dish. For samples that curled (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.

Furthermore, we developed three more systems of crosslinkers, growing and terminator hairpins by redesigning DNA sequence. Hydrogels with all four crosslink types swelled extensively in response to their corresponding hairpins but not to others. The fluorescently labeled hairpins intake experiments showed that fluorescently labeled hairpins accumulated in gels during the expansion process but not otherwise.

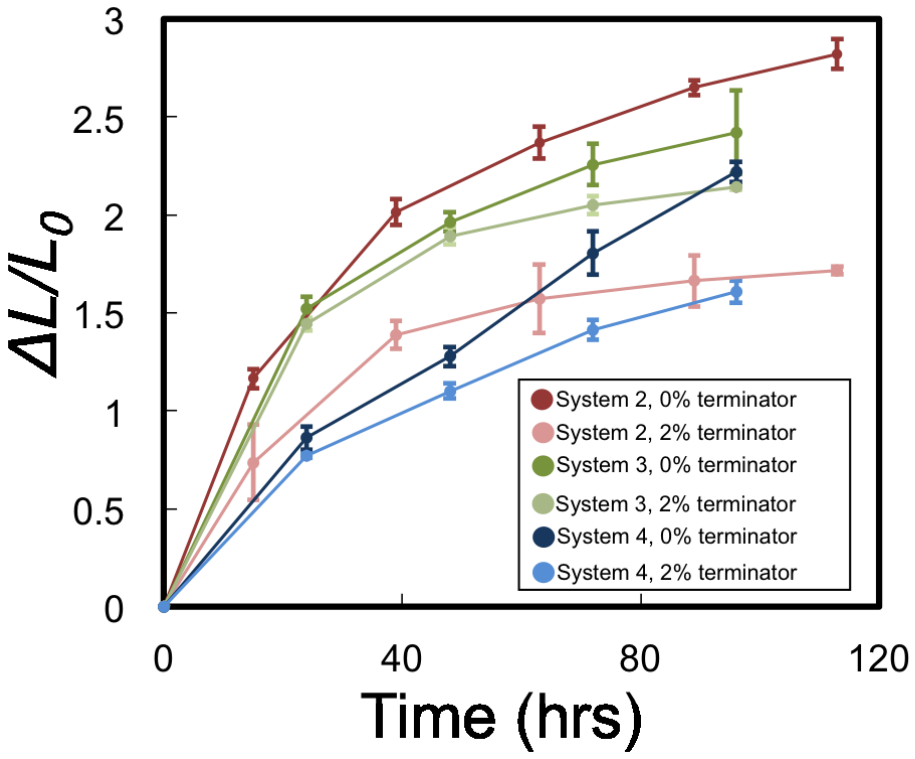

Figure 4 |DNA-driven expansion of poly(Am-co-DNA) gels crosslinked by different sequences in response to their respective polymerizing hairpins. Poly(Am-co-DNA) gel samples were prepared containing either system 2, system 3, or system 4 crosslink complexes. All gel samples were 60 µm x 1 mm x 1 mm in size. To visualize the gels, samples were stained overnight in 2x SYBR Green I nucleic acid stain and subsequently washed in fresh TAE/Mg2+ buffer. Gel samples were placed into 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 represent the standard deviation of the average swelling value of all the samples exposed to a given hairpin solution.

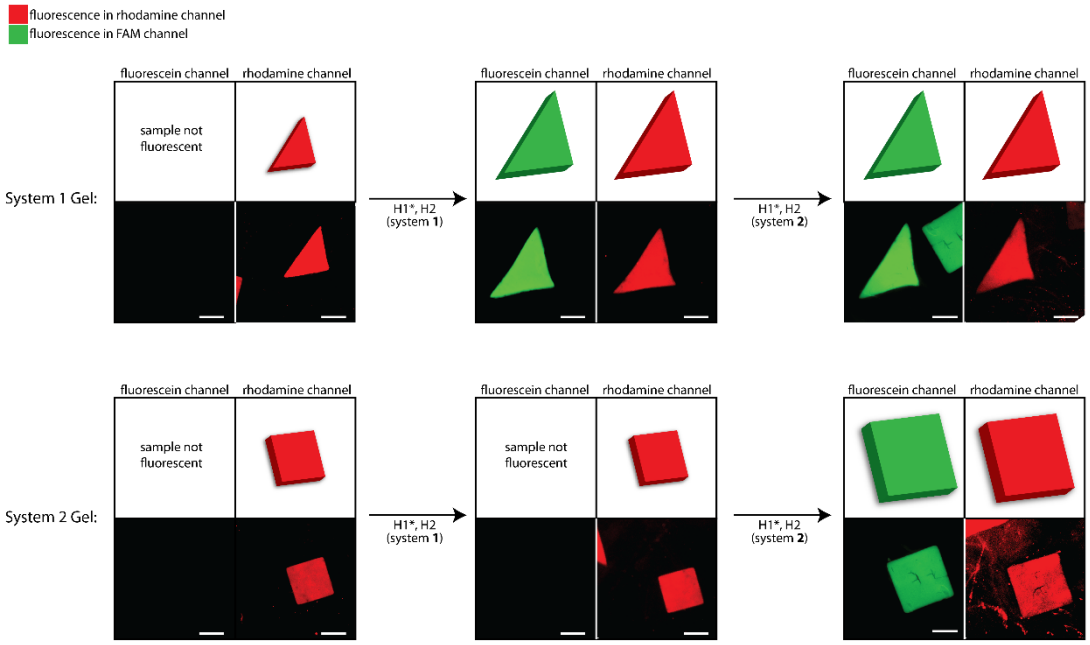

Figure 5 | Sequence-specific incorporation of hairpins into a poly(Am-co-DNA) gel. To verify that incorporation of DNA hairpins into a DNA copolymer gel is sequence specific, two DNA-*co*-Am-*co*-rhodamine gels – one crosslinked with system 1 and the other crosslinked with system 2 – were both sequentially treated with hairpins from each system. 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 grow and became visible in the FAM channel. Scale bars are 1 mm.

2.6 The Shape Change of Hydrogel Architectures

After we got enough information of the high-degree swelling of DNA-crosslinked monolayer hydrogel, we tried to build more complex structure that we can use to induce shape change with hairpins. Thus, we photopatterned beam-shaped hydrogel bilayers comprised of a 60 μm thick BIS-crosslinked polyacrylamide layer and a 60 μm thick System 1 DNA-crosslinked polyacrylamide layer. The beams curled slightly in DNA-free buffer due to different rates of solvent uptake by BIS-crosslinked and DNA-crosslinked gels. When bilayers were subsequently exposed to their corresponding hairpins, they curled much more tightly.

2.7 Modeling of Hydrogel Architectures

We applied finite element analysis to study bilayer folding caused by DNA-induced swelling. For the analysis, the bilayer structure was divided into discrete elements and the displacements at the element nodes were determined by solving the linear momentum balance for given boundary conditions and constitutive relations for the stress-strain-swelling behavior of the DNA- and BIS-crosslinked gels. Solvent diffusion occurs much more quickly through the micron-thick layers than the time-scale of folding and thus was neglected in the analysis. This allows us to apply a hyperelastic constitutive model that we developed previously to understand

chemomechanical shape change to describe the coupled DNA-driven swelling and stress response of the bilayers. Briefly, the stress response of the gel is assumed to be the sum of an elastic component for the entropic response of the polymer network, and the solvent pressure acting on the network. We used Flory-Huggins theory to describe the chemical potential of the water in the network that gives rise to the solvent pressure. We determined the final shape of the structure after DNA-driven swelling by changing the Flory-Huggins parameter in the DNA- and BIS-crosslinked gel layers to achieve the experimentally measured volumetric swelling ratios and then solving for the displacement field in the bilayer.

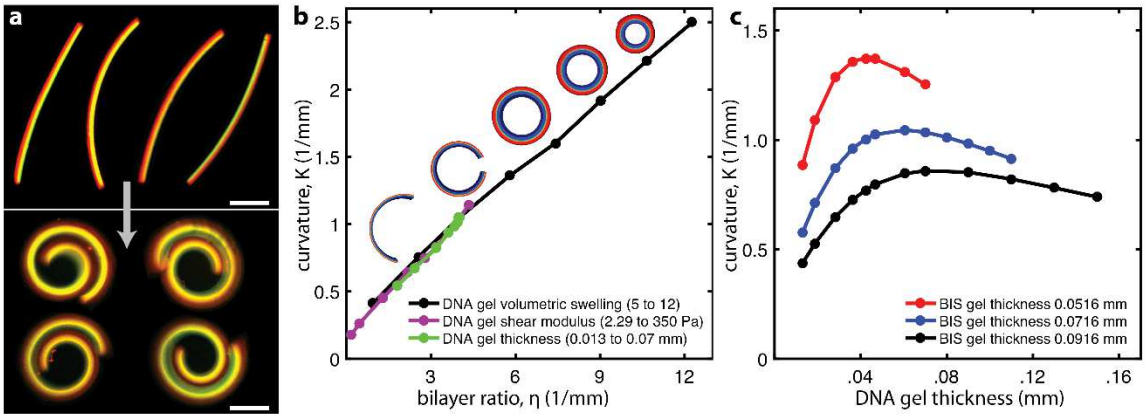

Figure 6 | Shape change driven by selective hydrogel swelling. (**a**) Side views of 4 different photopatterned hydrogel beams comprised of a 60 µm thick BIS-crosslinked polyacrylamide bottom layer (green), and a 60 µm thick DNA-crosslinked hydrogel layer (red). Top, before and bottom, after sequence-driven expansion of the DNA hydrogel layer (red). Scale bar is 1 mm. (**b**) Computational predictions of curvature resulting from DNA-driven actuation for a hydrogel bilayer consisting of BIS-crosslinked and DNA crosslinked layers. Visualization of curvature are shown. Bilayer size and properties are plotted as a function of bilayer ratio (Equation 1, main text) that is a function of the thicknesses, volumetric swelling ratio during actuation and the shear moduli after actuation of the two layers. The swelling ratio, shear modulus and thickness of the DNA layers were each varied keeping all other values constant. BIS gel shear modulus, layer thickness (0.0716 mm) and volumetric swelling ratio were kept constant. (**c**) Computational predictions of curvature change as a function of the thickness of the DNA and BIS gel layers. DNA gel layer thickness

was varied for a BIS gel layer with thicknesses of 0.0516 to 0.0916 mm. The shear moduli and swelling ratios are each at their measured or fit values.

For the model, we measured the Young's modulus of a BIS-crosslinked gel via an unconfined compression test as 2.2 kPa, which corresponds to a shear modulus of 733Pa assuming mechanical incompressibility. Because it was difficult and expensive to prepare and fully expand a DNA-crosslinked hydrogel via hairpin incorporation (in a 2% terminating hairpin solution) in sufficient volume to perform a compression test, we used the finite element model to fit the shear modulus of the DNA gel needed to obtain the curvature measured for the activated bilayer. The 229 Pa produced the best fit to the experimentally measured average curvature after DNA-driven expansion.

We used the model to investigate how DNA gel thickness, modulus, and the degree of expansion induced by DNA-driven swelling would be expected to control the final radius of curvature of DNA- and BIS-crosslinked gel bilayers. We varied them one at a time over a realistic range and predicted the final bilayer curvature for each case. We found that the results for all these different cases can collapsed onto a master curve, when the curvature is plotted as a function of the bilayer ratio η.

$$\eta = \frac{\frac{E_{DNA}t_{DNA}}{E_{BIS}t_{BIS}^2}\Delta\theta(1+\frac{t_{DNA}}{t_{BIS}})}{1+4(\frac{t_{DNA}}{t_{BIS}})(\frac{E_{DNA}}{E_{BIS}})+6(\frac{t_{DNA}}{t_{BIS}})^2(\frac{E_{DNA}}{E_{BIS}})+4(\frac{t_{DNA}}{t_{BIS}})^3(\frac{E_{DNA}}{E_{BIS}})+(\frac{t_{DNA}}{t_{BIS}})^4(\frac{E_{DNA}}{E_{BIS}})^2} \quad (1)$$

where E_{DNA} and E_{BIS} are the Young's moduli (Pa) of the DNA and BIS gels; t_{DNA} and t_{BIS} are the thicknesses (mm) of the DNA and BIS gels; Δ is the difference in the volumetric swelling ratio between the DNA and BIS gels. We found that if we

plot equilibrium bilayer curvature K (mm^{-1}) with the bilayer ratio η then all of the curves collapse on a straight line with the relation. Fitting to the line gives values of C=0.21 and K_0=0.2mm^{-1}, which was the initial bilayer curvature.

The parameter study showed that the curvature was more sensitive to the DNA gel swelling ratio, with which the curvature varied linearly, than to the shear modulus or thickness of the DNA gel layer, suggesting that the high degree of swelling achieved was instrumental in producing extensive shape change. The results also showed that increasing the thickness of the BIS-crosslinked gel layer lowered the curvature due to the increased flexural stiffness of BIS layer. However, the effect of varying the thickness of the DNA gel was more complicated. There was an optimum thickness of the DNA gel for which the curvature was maximized. The optimum DNA gel thickness increased with the BIS gel thickness. The parameter study predicts that that the high degree of swelling of the DNA gel allows very thick millimeter- to centimeter-sized bilayer structures to achieve large shape change (bending). For example, an initially flat 10 mm long by 7.23 mm-thick bilayer with the optimum DNA gel thickness and the maximum swelling ratio should fold into a complete circle after sequence specific DNA-triggered actuation.

2.8 Multi-step Actuation of Hydrogel Architectures

Finally, we explored how we can photopattern complex hydrogel architectures with different systems so that we can get actuation of different parts with different hairpins systems. We fabricated 6-petal flowers where two groups of petals responded

to two different sequences. When immersed in solution containing both types of hairpins, all of the petals folded. Exposure to just one type caused only the corresponding petals to fold, and petals could be folded in sequence through stepwise exposure to different hairpins solutions. We sometimes observed twisting of the petals during folding which we attribute to misalignment errors between layers during photopatterning. We further fabricated hydrogel “crab” devices, in which the antennae, claws and legs each curled in response to respective sequences, and found that we could direct shape change of the different domains programmability, both together and in different sequences. The structures remained in their actuated states for at least 60 days after they were removed from DNA-containing buffer.

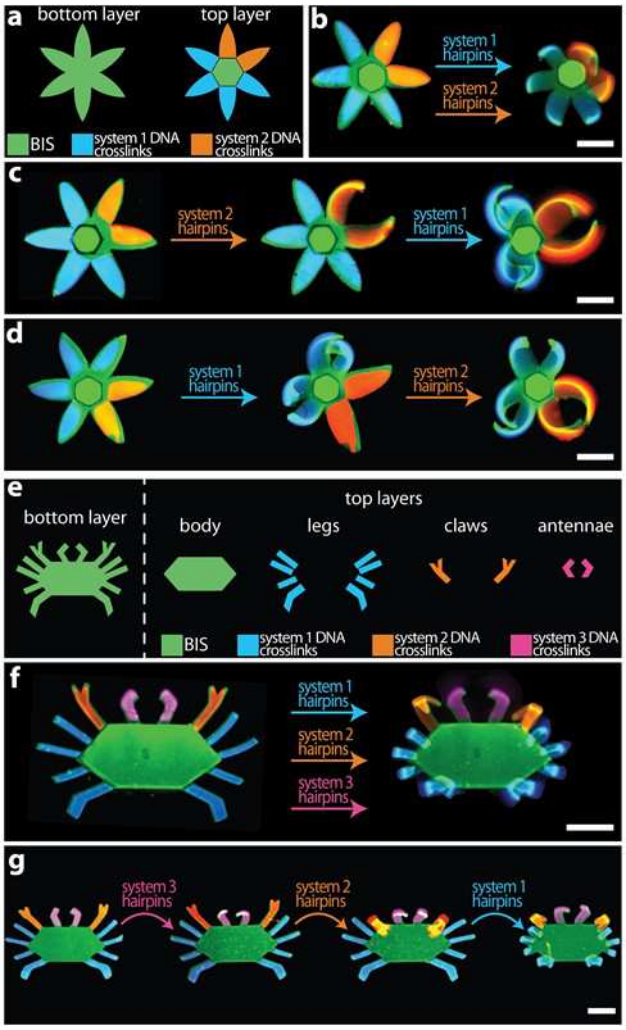

Figure 7 | DNA sequence-programmed shape change of macroscopic hydrogel shapes. (**a**) Schematic for a six-armed gripper comprised of a BIS-crosslinked domain (green) and DNA-crosslinked domains with System 1 (blue) and System 2 (red) crosslink sequences (see Methods). (**b**) All arms of the gripper in a curl in a solution of system 1 and 2 hairpins. (**c-d**) Only arms with the corresponding sequence are actuated in a solution of either system 1 (c) or system 2 hairpins. Exchanging the surrounding solution for one with other type of hairpins actuated the other set of arms. (**e**) Schematic for a hydrogel crab where the 2nd layer of the legs, claws and antennae are DNA crosslinked hydrogels with System 1 (blue), System 2 (red) and System 3 (purple) respectively. (**f**) Legs, claws and antennae actuate together in a solution of all hairpin types. (**g**) Exposing the structure to solution containing 1 hairpin type at a time through a series of solution exchanges leads to serial actuation. For all experiments shown, hairpin solutions contain 20 μM total concentration of each the hairpins, consisting of 98% polymerizing hairpins, 2% terminating hairpins. Scale bars for **b**, **c**, and **d** are 1mm. Scale bars for **f** and **g** are 2 mm.

3. Supplementary Information: DNA Crosslinked Hydrogel Architecture Shape-Change Driven By DNA Sequence

3.1 Supplementary Note S1: DNA Sequence List of Different Systems

System 1 Strands

DNA Strand	Sequence
C	**/5ACryd/TAAGTTCGCTGTGGCACCTGCACG**
C'	**/5ACryd/CAACGTGCAGGTGCCACAGCGTGG**
H1	**CCACGCTGTGGCACCTGCACGCACCCACGTGCAGGTGC CACAGCGAACTTA**
H2	**TGGGTGCGTGCAGGTGCCACAGCGTAAGTTCGCTGTGG CACCTGCACGTTG**
H1 Terminator ($H1_T$)	**CCACGCTGTGGCACCTGCACGTAGACTCGTGCAGGTGC CACAGCGAACTTA**
H2 Terminator ($H2_T$)	**TGGGTGCGTGCAGGTGCCACAGCGGCCTAGCGCTGTGG CACCTGCACGTTG**
H1_FAM	**/56-FAM/CCACGCTGTGGCACCTGCACGCACCCACGTGCAGG TGCCACAGCGAACTTA**
Control Hairpin	**GCTATCTAGCATCGCACGCTCTTTTTTGAGCGTGCGATG CTAGATGCGTAC**

System 2 Strand

DNA Strand	Sequence
C	**CTGTCTGCCTACCACTCCGTTGCG**
C'	**ATTCGCAACGGAGTGGTAGGCTTT**
H1	**AAAGCCTACCACTCCGTTGCGGAACCTCGCAACGGAGT GGTAGGCAGACAG**
H2	**AGGTTCCGCAACGGAGTGGTAGGCCTGTCTGCCTACCA CTCCGTTGCGTTG**

H1 Terminator ($H1_T$)	**AAAGCCTACCACTCCGTTGCGTCAAGCCGCAACGGAGTGGTAGGCAGACAG**
H2 Terminator ($H2_T$)	**AGGTTCCGCAACGGAGTGGTAGGCAATCGTGCCTACCACTCCGTTGCGTTG**
H1_FAM	**/56-FAM/AAAGCCTACCACTCCGTTGCGGAACCTCGCAACGGAGTGGTAGGCAGACAG**

System 3 Strands

DNA Strand	Sequence
C	**GGAACTCGGCAGTCGTCCAAGCGA**
C’	**ATCTCGCTTGGACGACTGCCGTAT**
H1	**ATACGGCAGTCGTCCAAGCGATACGGCTCGCTTGGACGACTGCCGAGTTCC**
H2	**GCCGTATCGCTTGGACGACTGCCGGGAACTCGGCAGTCGTCCAAGCGAGAT**
H1 Terminator ($H1_T$)	**ATACGGCAGTCGTCCAAGCGACTGAGTTCGCTTGGACGACTGCCGAGTTCC**
H2 Terminator ($H2_T$)	**GCCGTATCGCTTGGACGACTGCCGCAGATCCGGCAGTCGTCCAAGCGAGAT**

System 4 Strands

DNA Strand	Sequence
C	**ATCGGACCAGCACTTCGCCTACGG**
C’	**TGACCGTAGGCGAAGTGCTGGATG**
H1	**CATCCAGCACTTCGCCTACGGCTCTACCCGTAGGCGAAGTGCTGGTCCGAT**
H2	**GTAGAGCCGTAGGCGAAGTGCTGGATCGGACCAGCACTTCGCCTACGGTCA**

H1 Terminator ($H1_T$)	**CATCCAGCACTTCGCCTACGGAAGGTGCCGTAGGCGAAGTGCTGGTCCGAT**
H2 Terminator ($H2_T$)	**GTAGAGCCGTAGGCGAAGTGCTGGTGTATGCCAGCACTTCGCCTACGGTCA**

Supplementary Table 1: DNA Sequences

3.2 Supplementary Note S2: Photopatterning Process Diagram of Structures

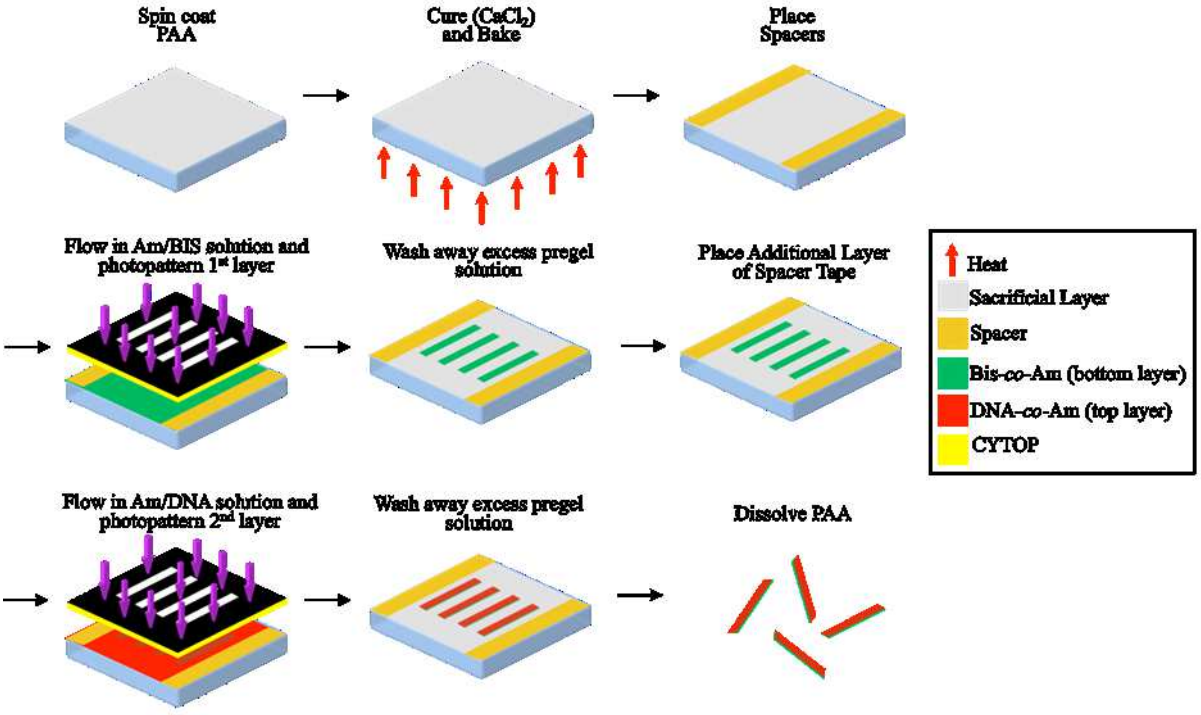

Figure 8-1 | Process Diagram for Am-co-BIS / Am-co-DNA Hydrogel Bilayer Fabrication. Parameters such as bake temperature/time, spacer thickness and solution concentrations are listed before in Methods. After each UV exposure, the resulting samples were washed with TAE/Mg^{2+}/Ca^{2+} buffer to remove unpolymerized monomers and DNA crosslinks. The calcium cations in the buffer prevent degradation of the ionic crosslinks of the poly(acrylic acid) sacrificial layer.

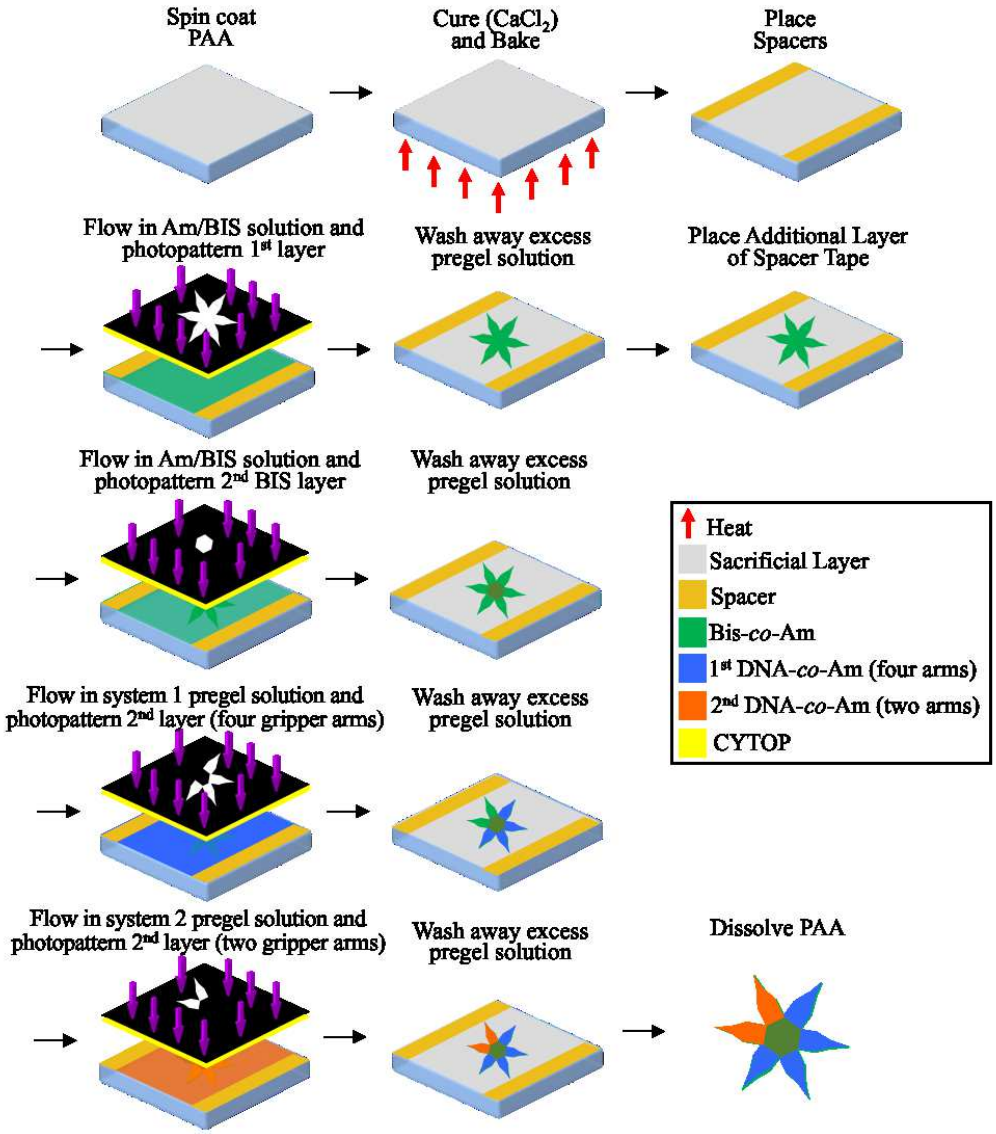

Figure 8-2 | Process Diagram of Flower Bilayer Fabrication.

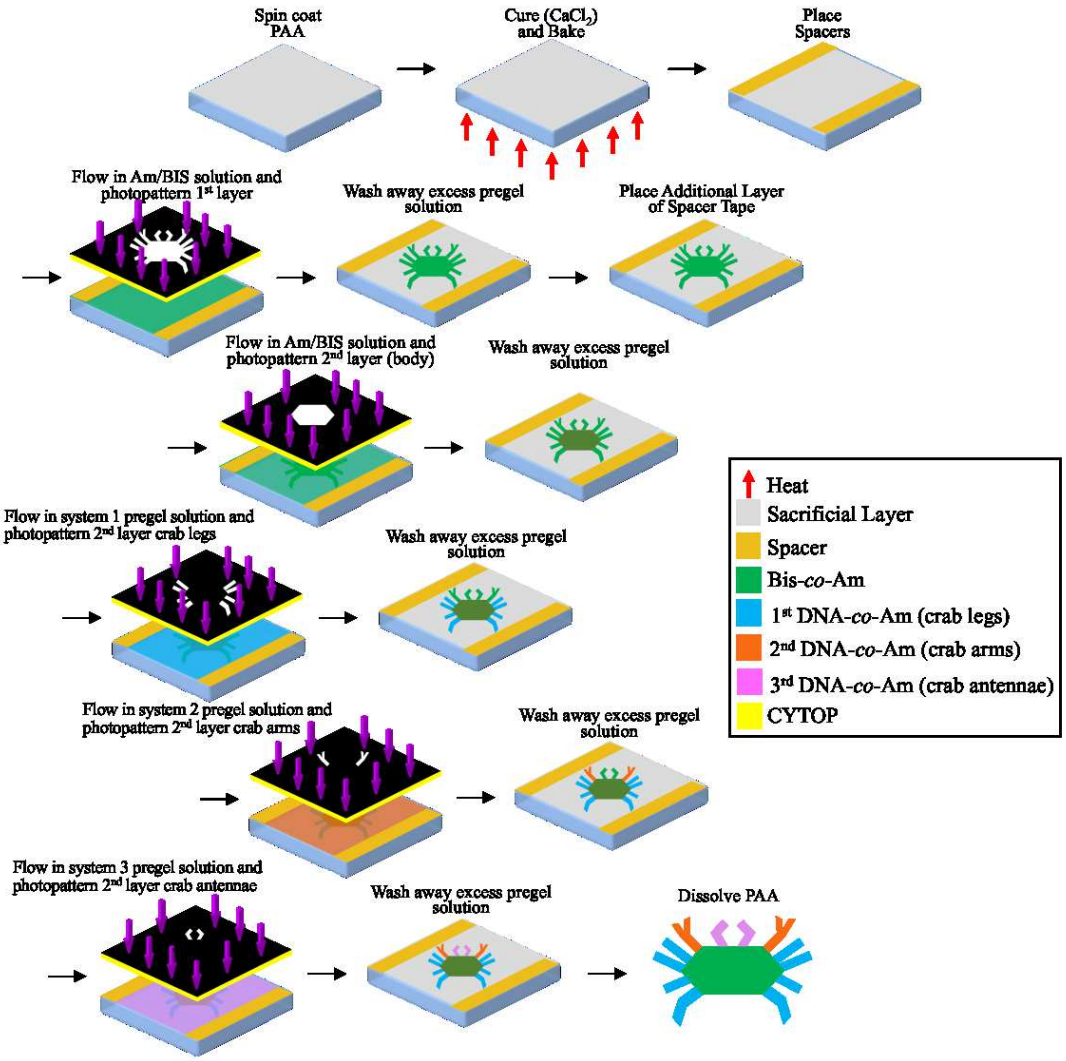

Figure 8-3 | Process Diagram of Crab Bilayer Fabrication.

4. DNA Crosslinked Microgel via Microfluidic

4.1 Introduction

Environment responsive smart microgels are soft material particles being able to react to environmental change. Those smart microgels are able to swell, shrink or melt away with changes of pH, temperature or light exposure. [23-25]The ability to response to environment of those microgels show great potentials in fields such as tissue engineering, catalysis and drug delivery. In recent years, microfluidic droplets formation has become a powerful technique to generate microgel particles. The technique produce highly monodisperse microgels with controllable size. We can fabricate droplets minimum to 10μm with microfluidic junction.[25-27]

In previous research, to make smart microgel, people use stimuli sensitive polymer systems. For example, poly (N-isopropylacrylamide) (pNIPAAm) has attracted many researchers for its great thermal behavior to make temperature sensitive microgel.[28] Poly acrylic acid (PAA) and poly acrylamide (PAAm), with ionic pendant group in polymer backbone, can be used for pH-responsive microgel. [29] However, non-specific information as pH and temperature has its own limitation. If we want to further build a range of smart microgels response to different signals, we need more signal choices without affecting each other. In this case, stimuli as pH and temperature are not enough to achieve higher complexity.

DNA, as natural biological polymer, has been applied as fundamental units for microscale and nanoscale material engineering in recent decades. The unique advantage of DNA molecular is the high sequence dependency of DNA hybridization. With DNA nanotechnology developing rapidly, several groups has developed DNA hydrogel and DNA crosslinked hydrogel, which show many interesting properties.

Instead of using pH and temperature, a good strategy to achieve the stimulus-response system is to apply DNA reaction into microgel system. To make the microgel responsive to DNA, the first part is to pre-crosslink DNA into polymer network. In previous research, N,N′-methylenebiscarylamide (BIS) crosslinker was often reacted with acrylamide monomer for gelation. In this case, we replaced the BIS crosslinkers with DNA crosslinkers.[5, 30] Those DNA crosslinkers, with active binding region allow further DNA hybridization chain polymerization (HCR).[31] The second step is to trigger the DNA crosslinkers with DNA hairpin inputs.[32] With the length increments of DNA crosslinkers, the polymer network extended therefore the hydrogel swelled. The advantage of the system is specificity that one kind of crosslinker only reacts with one kind of hairpin input highly dependent on DNA sequence.

In this section, we use microfluidic system to generate monodisperse PAAm microgel from acrylamide monomer.[27] We apply DNA crosslinker to the microgel, and trigger the swelling process of microgel with DNA inputs. We present the swelling kinetics of the microgel and the morphology change of the process. We show

that with the application of DNA crosslinker, the smart microgel is able to respond to specific DNA sequence signal.

4.2 Pre-gel Solution Preparation

The BIS-crosslinked pre-gel solution was prepared by mixing MilliQ water, TAE/Mg2+ buffer, 40 % (w/v) 19:1 Am:BIS (BioRad Laboratories, Inc., catalog #1610144), Irgacure 2100(Ciba) and methacryloxyethyl thiocarbamoyl rhodamine B (Polysciences, Inc., catalog #23591). To make TAE/ Mg2+ buffer, we supplemented 12.5 mM magnesium acetate tetrahydrate (Sigma #228648) with 1×TAE buffer (40 mM tris-acetate, 1 mM EDTA) that had been previously diluted from 50x stock (Life Technologies, Catalog #24710-030). The final concentrations of the other species is as follows: 10% (w/v) of 19:1 Am:BIS, 10% (v/v) Irgacure 2100, and 2.99 mM methacrylated rhodamine B. After mixing by ultra-sonification for 2 minutes, the solution was degassed under vacuum for 15 minutes and sealed by Nitrogen to minimize the concentration of O2 in solution. To emulsify aqueous droplets in microfluidic channel, we used hexane (Sigma #296090) as our organic phase. The use of hexane instead of mineral oil simplified the extraction and washing processes. 1% Span 80 (Sigma # S6760) was mixed into hexane as surfactant. We then bubbled Nitrogen into solution for 15 minutes to degas O2 in hexane.

DNA Sequence List.

Name	**Sequence**
A1	**TAAGTTCGCTGTGGCACCTGCACG**

R1	**AACGTGCAGGTGCCACAGCGTG**
H1	**CACGCTGTGGCACCTGCACGCACCCACGTGCAGGTGC CACAGCGAACTTA**
H2	**TGGGTGCGTGCAGGTGCCACAGCGTAAGTTCGCTGTG GCACCTGCACGTT**

Apart from BIS crosslinker pre-gel solution, we further make DNA crosslinker pre-gel solution. To make DNA crosslinker pre-gel solution, the crosslinker DNA double strands (referred as A&R strand), modified with Acrydite, were prepared ahead by dissolving lyophilized DNA samples to a final concentration of approximately 25 mM in 1×TAE/Mg2+ buffer. We verified the oligonucleotide concentrations with absorbance spectroscopy at 260 nm. The DNA crosslinkers were diluted with MillQ water and TAE/Mg2+ buffer, then incubated at 90 °C for 5 minutes, followed by anealing the solution from 90 °C to 20 °C at 1 °C per minute for crosslinkers to hybridize. Fresh acrylamide (Bio-Rad Catalog #161-0100), Irgacure2100 and methacrylated rhodamine B were added right before polymerization. The final concentration of all components of DNA-crosslinked pre-gel solution shows as allow, 2.3 mM of DNA A&R strands, 1×TAE/Mg2+ buffer, 10% (v/v) Irgacure 2100, and 2.99 mM methacrylated rhodamine B.

4.3 Microfluidic Fabrication

The microfluidic fabrications were operated in Nitrogen atmosphere. The monomer solution was loaded in 100 μL glass syringe (Sigma Aldrich #509469). The flow rates of both phase were controlled with syringe pump (New Era Pump Systems, NE300). As sketched on Figure-1, we emulsified droplets with T-junction chip

(Dolomite #3000437). The size of droplets was controlled by the flow rate of both aqueous phase and oil phase flow. As for the flow rate, we used 5 μL/min for the aqueous phase and 15 μL/min for the organic phase solution in our study.

After the droplets formation, microgels are obtained by UV polymerization. The polymerization of micro-droplets requires much more UV-dose than a bulk of solution, and in this case we polymerized for 20 minutes with 365 nm UV light (Neutronix Quintel aligner) at an intensity of 7.55 mW/cm2 to form microgels.

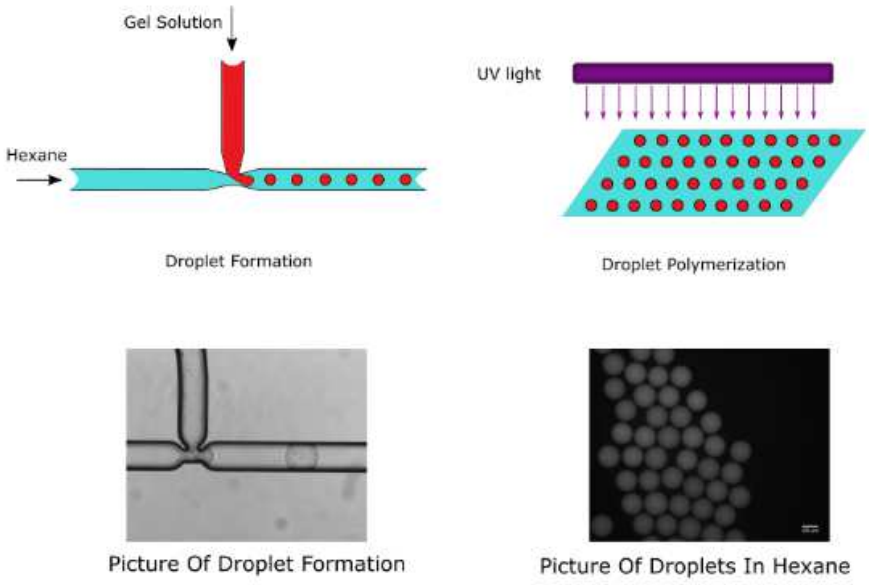

Figure 9 | Microfluidic Fabrication Process of BIS-crosslinked PAAm Microgels. We emulsified the droplets in T-junction, collected them and then polymerized them with UV light. All steps are operated in Nitrogen environment. The scale bar in pictures is 200 μm.

To evaluate the quality of the products, we measured the roundness and the diameter of the BIS-crosslinked microgels. The average roundness of BIS-crosslinked microgels is 0.946(±0.032) and the average diameter is 104.95(±9.88) μm. With the same flowrate, however, the DNA-crosslinked microgels appeared to have bigger

size. The average size of the DNA crosslinked microgel reached 184.45(±17.00) μm. The result may possibly due to the stronger water intake capacity of the DNA-crosslinked hydrogel or the larger size of the DNA crosslinker.

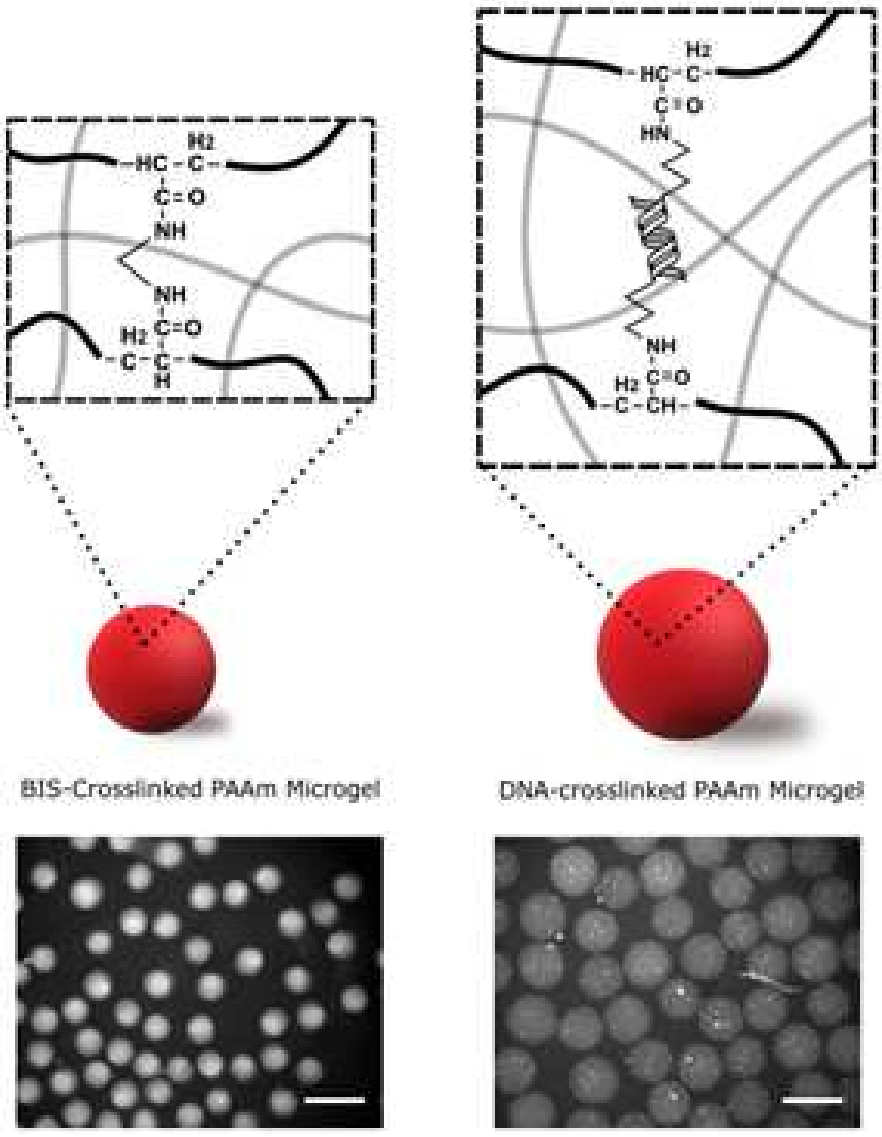

Figure 10 | Fabricated BIS-crosslinked PAAm microgels and DNA-crosslinked microgels. The scale bar in both pictures are 500 μm. The real size of DNA crosslinker is much larger than what we draw in the chart.

4.4 Swelling of Microgel Triggered by DNA

To trigger the swelling process of formed DNA-crosslinked microgel, we added corresponding DNA hairpin strands to the surroundings of microgels. The DNA hairpin double strands, referred as H1 and H2, were separately prepared by incubating at 95 °C for 5 minutes, followed by flash annealing in ice for 2 minutes. The final concentration of both H1 and H2 in solution is 20 μM. Once the hairpins were added into the microgel surroundings, the DNA-crosslinked microgel would start swelling. The mechanism of the swelling process with DNA hybridization chain polymerization is illustrated in Figure-2. Step by step, H1 and H2 bound with the A&R crosslinkers between polymer chains, therefore extended the polymer network to achieve tremendous expansion of hydrogel. After swelling in 20μM hairpins solution for 10 hours, the microgels swelled from around 200 μm to 400 μm in diameter, equivalent to 8 times increase in volume. (Figure-3). As presented in Figure 3, the microgel showed linear growth in diameter. The rate of growth is around 30 μm per hour. The microgel still grew after 10 hours. As the crosslink density goes down, the microgel was not able to hold itself and became invisible in the end.

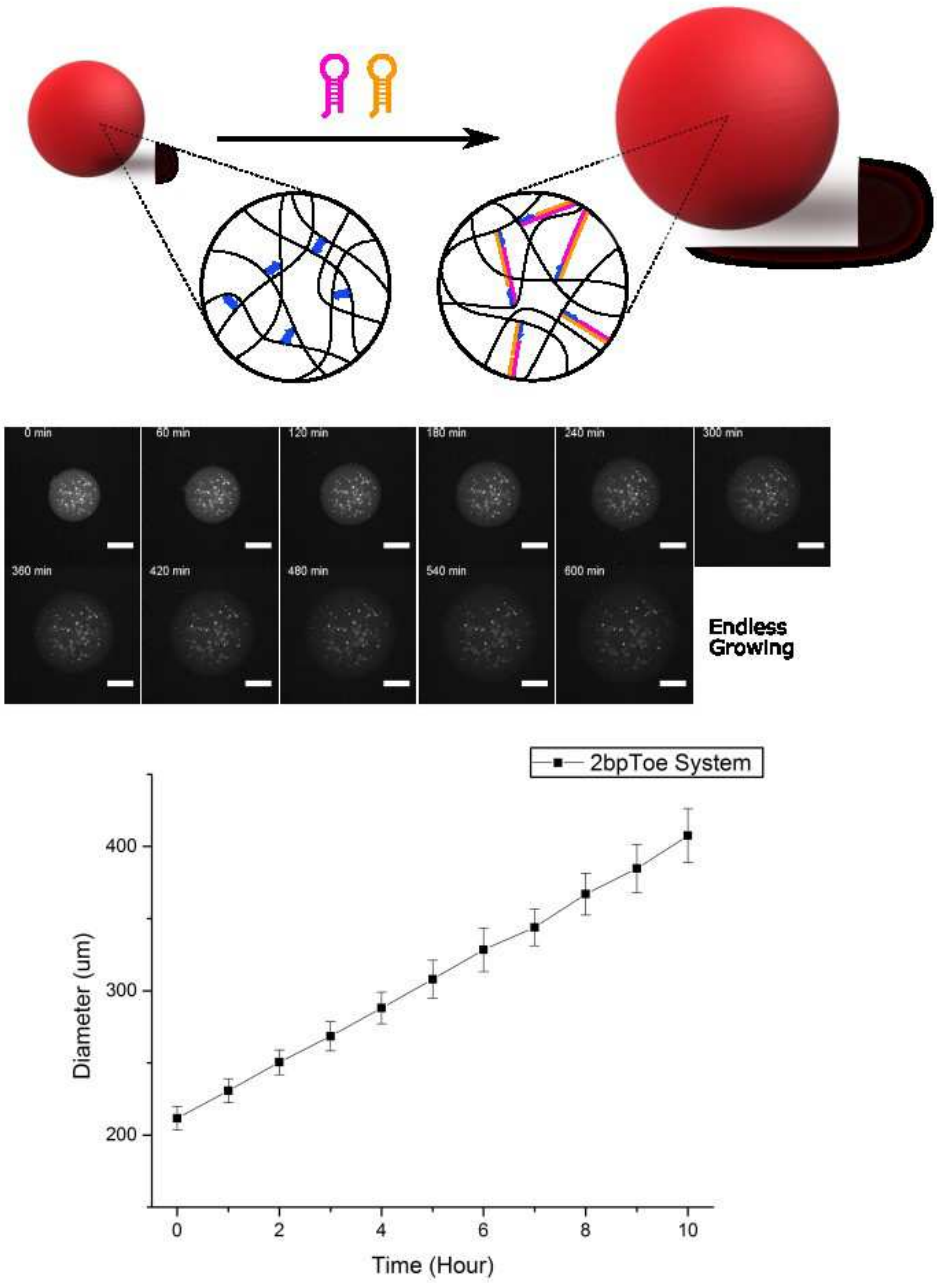

Figure 11 | The swelling process triggered by DNA hairpin. (A)Scheme of DNA triggered swelling process of DNA-crosslinked hydrogel. (B) Time lapse swelling images of microgel. The scale bar of all pictures is 200 μm (C) Rate of swelling of microgels. Hairpin concentration: 20 μM.

5. Conclusion and Future Work

5.1 Conclusion

The objective and motivation of this thesis is to develop a new hydrogel responsive to specific DNA sequence, and to apply this new material to various applications. By copolymerizing DNA crosslinkers into polyacrylamide network via photo polymerization, we can get a new kind of DNA crosslinked hydrogel that can be triggered by corresponding DNA sequence signals. With this new material, we are able to build soft material architectures that can be actuated by DNA with a designed sequential order. This technology inaugurates a new signal-response system inside the soft material architectures, or soft robots. With the particularity of the DNA signal, this technology can be used in complex soft architectures with multiple parts to actuate. Also, as we fabricate the DNA crosslinked microgel with microfluidic device, we see the potential of the application of the hydrogel in drug delivery and tissue engineering. If the microgel only swell with the responsive DNA sequence, we can further modify the DNA to let it responsive to a series of small molecular signals, which will be of great use in sensing and drug releasing in the near future.

Chapter 2 describes the new process for photopatterning DNA co-polymer hydrogel architectures containing hydrogel domains selectively responsive to multiple DNA sequences. The resulting millimeter- to centimeter-scale devices undergo different dramatic shape changes in response to different biomolecular inputs. We

characterize the mechanical properties and shape change behavior using experiments and finite element simulations. We put some supplementary information in Chapter 3.

Chapter 4 describes the process of making monodisperse DNA crosslinked microgel via microfluidic. With the new protocol, we can fabricate uniform-sized DNA crosslinked microgel in a rapid and easy way. These DNA crosslinked microgel can swell dramatically once triggered. The new kind of smart microgel introduce a new trigger to the stimuli-response system for smart microgel.

5.2 Future Work

The material shows great potential in soft robots and microcapsule drug delivery. However, there are still some parts of this material to be improved. One of the most important improvement to make is the reversibility of the DNA trigger. As for other stimuli used for actuating soft architectures as pH and temperature, those stimuli are reversible so that the soft material can swell and shrink and the architectures can be actuated then switch back to initial state. But our DNA stimuli-response system is forward driven and irreversible, which limits the future use for the DNA crosslinked hydrogel. So we try to redesign the system, and hopefully to show some reversibility.

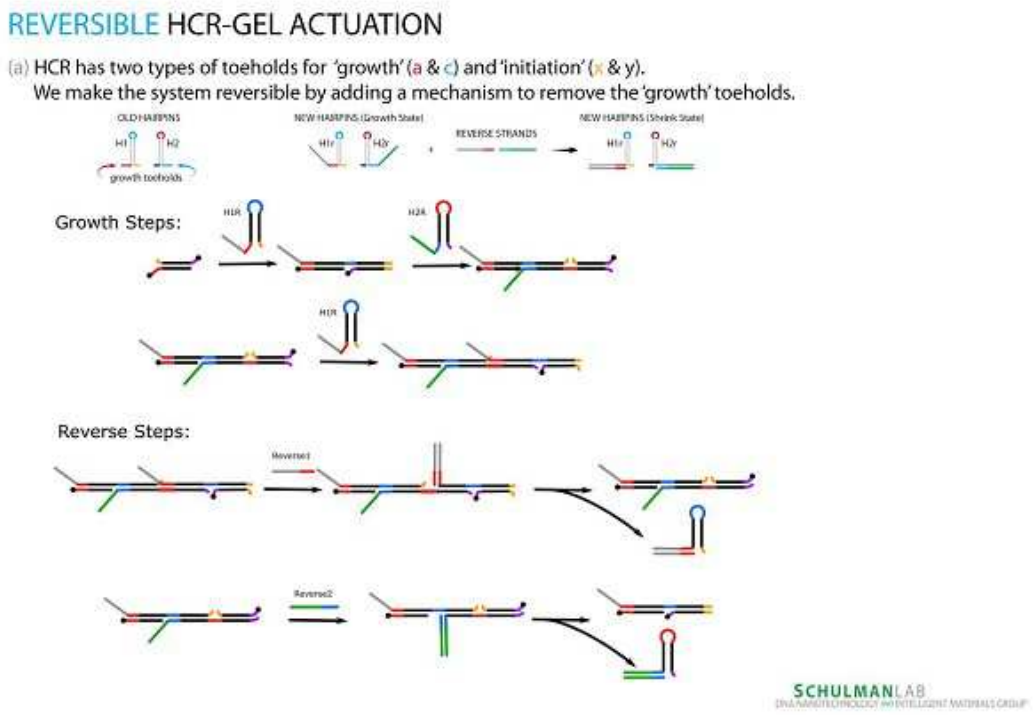

Figure 12 | Design of reversible hairpin

In Figure 12, we designed new hairpins to trigger the swelling process. Comparing to the old hairpins, we add 15 bases before the toehold. In the forward polymerizing reaction, the new hairpins play the same roles as the old ones. However, with the added part, we can use the reverse strands to remove the H1r and H2r hairpins away from the polymerized strand. If this mechanism works in our system, we will be able to shrink the hydrogel back to its initial state. Although there are still other problems to fix, but the material will be of greater use if we can figure out the reversibility problem.

Reference

1. Zhang, D. Y.; Seelig, G., Dynamic DNA nanotechnology using strand-displacement reactions. *Nature chemistry* **2011,** *3* (2), 103-113.
2. Tørring, T.; Voigt, N. V.; Nangreave, J.; Yan, H.; Gothelf, K. V., DNA origami: a quantum leap for self-assembly of complex structures. *Chemical Society Reviews* **2011,** *40* (12), 5636-5646.
3. Seeman, N. C., From genes to machines: DNA nanomechanical devices. *Trends Biochem Sci* **2005,** *30* (3), 119-25.
4. Rothemund, P. W., Folding DNA to create nanoscale shapes and patterns. *Nature* **2006,** *440* (7082), 297-302.
5. Kang, H.; Liu, H.; Zhang, X.; Yan, J.; Zhu, Z.; Peng, L.; Yang, H.; Kim, Y.; Tan, W., Photoresponsive DNA-cross-linked hydrogels for controllable release and cancer therapy. *Langmuir* **2010,** *27* (1), 399-408.
6. Zhou, W.; Huang, P.-J. J.; Ding, J.; Liu, J., Aptamer-based biosensors for biomedical diagnostics. *Analyst* **2014,** *139* (11), 2627-2640.
7. Seeman, N. C., The use of branched DNA for nanoscale fabrication. *Nanotechnology* **1991,** *2* (3), 149.
8. Simmel, F. C., Processive motion of bipedal DNA walkers. *ChemPhysChem* **2009,** *10* (15), 2593-2597.
9. Bath, J.; Turberfield, A. J., DNA nanomachines. *Nature nanotechnology* **2007,** *2* (5), 275-284.
10. Shepherd, R. F.; Ilievski, F.; Choi, W.; Morin, S. A.; Stokes, A. A.; Mazzeo, A. D.; Chen, X.; Wang, M.; Whitesides, G. M., Multigait soft robot. *Proceedings of the National Academy of Sciences* **2011,** *108* (51), 20400-20403.
11. Plamper, F. A.; Richtering, W., Functional Microgels and Microgel Systems. *Accounts of Chemical Research* **2017,** *50* (2), 131-140.
12. Oh, J. K.; Lee, D. I.; Park, J. M., Biopolymer-based microgels/nanogels for drug delivery applications. *Progress in Polymer Science* **2009,** *34* (12), 1261-1282.
13. Murthy, N.; Xu, M.; Schuck, S.; Kunisawa, J.; Shastri, N.; Fréchet, J. M., A macromolecular delivery vehicle for protein-based vaccines: acid-degradable protein-loaded microgels. *Proceedings of the National Academy of Sciences* **2003,** *100* (9), 4995-5000.
14. Ionov, L., Biomimetic Hydrogel‐Based Actuating Systems. *Advanced Functional Materials* **2013,** *23* (36), 4555-4570.
15. Lin, D. C.; Yurke, B.; Langrana, N. A., Mechanical properties of a reversible, DNA-crosslinked polyacrylamide hydrogel. *Journal of biomechanical engineering* **2004,** *126* (1), 104-110.
16. Lee, J. B.; Peng, S.; Yang, D.; Roh, Y. H.; Funabashi, H.; Park, N.; Rice, E. J.; Chen, L.; Long, R.; Wu, M.; Luo, D., A mechanical metamaterial made from a DNA hydrogel. *Nat Nanotechnol* **2012,** *7* (12), 816-20.

17. Jin, J.; Xing, Y.; Xi, Y.; Liu, X.; Zhou, T.; Ma, X.; Yang, Z.; Wang, S.; Liu, D., A triggered DNA hydrogel cover to envelop and release single cells. *Adv Mater* **2013,** *25* (34), 4714-7.
18. Liu, Y.; Boyles, J. K.; Genzer, J.; Dickey, M. D., Self-folding of polymer sheets using local light absorption. *Soft Matter* **2012,** *8* (6), 1764-1769.
19. Postma, S. G.; Vialshin, I. N.; Gerritsen, C. Y.; Bao, M.; Huck, W. T., Preprogramming complex hydrogel responses using enzymatic reaction networks. *Angewandte Chemie International Edition* **2017,** *56* (7), 1794-1798.
20. Zarzar, L. D.; Aizenberg, J., Stimuli-responsive chemomechanical actuation: a hybrid materials approach. *Accounts of chemical research* **2013,** *47* (2), 530-539.
21. Flory, P. J.; Rehner Jr, J., Statistical mechanics of cross-linked polymer networks I. Rubberlike elasticity. *The Journal of Chemical Physics* **1943,** *11* (11), 512-520.
22. Zhang, Y.; Zhao, Y.-p., Applicability range of Stoney's formula and modified formulas for a film/substrate bilayer. *Journal of applied physics* **2006,** *99* (5), 053513.
23. Klinger, D.; Landfester, K., Stimuli-responsive microgels for the loading and release of functional compounds: Fundamental concepts and applications. *Polymer* **2012,** *53* (23), 5209-5231.
24. Jiang, Y.; Chen, J.; Deng, C.; Suuronen, E. J.; Zhong, Z., Click hydrogels, microgels and nanogels: emerging platforms for drug delivery and tissue engineering. *Biomaterials* **2014,** *35* (18), 4969-4985.
25. Duncanson, W. J.; Lin, T.; Abate, A. R.; Seiffert, S.; Shah, R. K.; Weitz, D. A., Microfluidic synthesis of advanced microparticles for encapsulation and controlled release. *Lab Chip* **2012,** *12* (12), 2135-45.
26. Seiffert, S.; Weitz, D. A., Microfluidic fabrication of smart microgels from macromolecular precursors. *Polymer* **2010,** *51* (25), 5883-5889.
27. Kim, J. W.; Utada, A. S.; Fernández-Nieves, A.; Hu, Z.; Weitz, D. A., Fabrication of monodisperse gel shells and functional microgels in microfluidic devices. *Angewandte Chemie* **2007,** *119* (11), 1851-1854.
28. Seiffert, S.; Thiele, J.; Abate, A. R.; Weitz, D. A., Smart microgel capsules from macromolecular precursors. *Journal of the American Chemical Society* **2010,** *132* (18), 6606-6609.
29. Cheng, E.; Xing, Y.; Chen, P.; Yang, Y.; Sun, Y.; Zhou, D.; Xu, L.; Fan, Q.; Liu, D., A pH-triggered, fast-responding DNA hydrogel. *Angew Chem Int Ed Engl* **2009,** *48* (41), 7660-3.
30. Xing, Y.; Cheng, E.; Yang, Y.; Chen, P.; Zhang, T.; Sun, Y.; Yang, Z.; Liu, D., Self-assembled DNA hydrogels with designable thermal and enzymatic responsiveness. *Adv Mater* **2011,** *23* (9), 1117-21.
31. Dirks, R. M.; Pierce, N. A., Triggered amplification by hybridization chain reaction. *Proceedings of the National Academy of Sciences of the United States of America* **2004,** *101* (43), 15275-15278.
32. Green, S. J.; Lubrich, D.; Turberfield, A. J., DNA hairpins: fuel for autonomous DNA devices. *Biophysical journal* **2006,** *91* (8), 2966-2975.

Curriculum Vitae

Education

M.S

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Professional Appointments

Teaching Assistant, Department of Mechanical Engineering

2016 • 530.633 Mechanics of the Biological Systems and Biophysical Methodologies

Peer Reviewed Publications

1. Cangialosi, A; Yoon, C; Liu, J; **Huang, Q**; Guo, J; Nguyen, T; Gracias, D; Schulman, R; Programmable DNA Sequence-Driven Shape Change of Photopatterned Hydrogels via High-Degree Swelling, *Science*, in revision.
2. **Huang, Q**; Schulman, R; Specific DNA Sequence Responsive Smart Microgel via Microfluidic Fabrication, in preparation.

Honors and Awards

2016 Johns Hopkins ChemBE Master's Essay Scholarship