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Supplementary Information for

Swelling characteristics of DNA polymerization gels

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Materials and Methods

Chemicals and DNA

Polyethylene glycol diacrylate M_n 10,000 (PEGDA10k) was obtained from Sigma-Aldrich (Cat. No. 729094). The fluorophore RhodamineB-methacrylate was purchased from PolySciences, Inc. (Cat. No. 25404-100) and was used to visualize the hydrogels. Acrylamide (Bio-Rad, Cat. No. 161-0100) was solubilized in MilliQ purified water. The UV-sensitive initiator Omnirad 2100 (formerly known as Irgacure 2100, iGM Resins USA, #55924582) photoinitiator was used to polymerize hydrogels. All DNA strands were purchased with standard desalting purification from Integrated DNA Technologies, Inc. Acrydite-modified strands were solubilized using 1x TAE buffer (Life Technologies, Cat. No. 24710-030) supplemented with 12.5 mM magnesium acetate tetrahydrate (Sigma-Aldrich, Cat. No. M5661). All unmodified DNA strands were solubilized using MilliQ purified water. DNA sequences were adapted from previous literature[1–3] or designed using NUPACK[4] as previously described[2] and are listed in Supplementary Table 1.

Preparation of DNA complexes

DNA crosslink complexes were annealed in 1x TAE buffer supplemented with 12.5 mM magnesium acetate tetrahydrate (TAEM) from 90 to 20 °C in an Eppendorf PCR at 1 °C/minute at a concentration of 3 mM per strand. Hairpin-forming strands were heated to 95 °C for 15 minutes at a concentration of 200 or 600 μM, followed by flash cooling on ice for 3 minutes.

Preparation of poly(PEGDA10k-*co*-DNA) pre-gel solution

PEGDA10k powder was mixed with MilliQ purified water and 10x TAEM. After the PEGDA10k was fully dissolved, acrydite-modified DNA (3 mM), RhodamineB-methacrylate (29.9 mM), and Omnirad 2100 (75% v/v in butanol) were mixed into the solution. The final

concentrations were 10% w/v PEGDA10k, 2.74 mM RhodamineB, and 3% v/v Irgacure 2100. Unless noted otherwise, the final concentration of DNA strands or complexes was 1.154 mM. After mixing with a pipette, the pre-gel solution was sonicated for 10 minutes and degassed for 15 minutes.

Synthesis of poly(PEGDA10k-*co*-DNA) hydrogel triangles

We assembled photolithography chambers, as reported previously.[1,5] To pattern equilateral triangle-shaped DNA hydrogels, we designed triangle-shaped masks with 1mm side-length using AutoCAD and made the Cr masks using the method reported.[1,5] The thickness of the patterned hydrogel could be tuned using different thicknesses of spacers (160 µm in this paper unless otherwise stated). The pre-gel solution was injected into the photo patterning chamber and then exposed to a 365 nm UV light source (Neutronix Quintel aligner) with an energy dose of 600 mJ/cm^2. The chamber was gently disassembled after the polymerization. We use TAEM to wash the extra pre-gel solution and hydrate the gel. The hydrogel was then stored in the TAEM at 4 °C to achieve complete hydration until use; the portion of intrinsic swelling with TAEM was not included in the swelling kinetics calculations.

Preparation of poly(Am-*co*-DNA) pre-gel solution

Acrylamide, acrydite-modified DNA (3 mM), RhodamineB-methacrylate (29.9 mM), and Irgacure 2100 (75% v/v in butanol) were mixed into the solution. The final concentrations were 10% w/v PEGDA10k, 2.74 mM RhodamineB, and 3% v/v Irgacure 2100. The final concentration of DNA strands or complexes was 1.154 mM unless noted otherwise. After mixing with a pipette, the pre-gel solution was sonicated for 10 minutes and degassed for 15 minutes.

Synthesis of hydrogel particles

DNA-integrated polyacrylamide and PEGDA10k hydrogel particles were prepared as previously described.[2] Hydrogel droplets were polymerized in mineral oil with 365 nm light using a Benchtop 3UV Transilluminator (UVP) for 1.5 minutes. Polymerized particles were purified from the mineral oil and stored in 1x TAEM at room temperature or 4°C until use.

Swelling DNA-integrated hydrogels

Hydrogel swelling experiments were conducted with one hydrogel per well in 96-well plates (Fisher Scientific). Unless noted otherwise, hydrogels were expanded in TAEM supplemented with 0.001% v/v Tween20 to prevent hydrogel from sticking to the well's surface. Hairpins were added such that at least 60 µL of the 100 µL total in each well was TAEM with Tween20, and the remaining solution was the hairpin stock solution. Following the addition of hairpin solutions to each well, we utilized a pipette set to a volume of 90 µL and thoroughly mixed the solution by performing at least 10 times repeatedly dispensing and withdrawing. Images were captured every 30 minutes using a humidified Syngene G:Box EF2 gel imager equipped with a blue light transilluminator (Clare Chemical, Em. max ~450 nm) and a UV032 filter (Syngene, bandpass 572-630 nm) or on an Olympus IX73 fluorescence microscope.

Experiments comparing hydrogel expansion in different buffer/salt conditions

Hydrogel swelling experiments were conducted with one hydrogel per well in 96-well plates (Fisher Scientific). The volume of liquid in each well was 100 µL. The stock concentrations of buffers were 5x for SPSC and 10x for PBS, TAE/Mg^{2+}, TAE/Na^{+}, and TAE/Mg^{2+}/Na^{+}. The concentrations of species in each 1x buffer are listed in Supp. Table 2.

For poly(Am-*co*-S1dsA^{ac}R^{ac}1.154) hydrogel particles, the hairpin stock solutions were at 400 µM per hairpin and were snap cooled in 1x TAEM. The hairpin concentration during hydrogel

expansion was initially 20 μM per hairpin. The reaction volume for 10x buffers consisted of 10 μL of 10x buffer, 80 μL MilliQ water, and 5 μL each hairpin stock solution. The reaction volume for 5x buffers consisted of 20 μL of 5x buffer, 70 μL MilliQ water, and 5 μL each hairpin stock solution. Since the hairpins were snap cooled in 1x TAEM, the final buffer conditions in each condition included an additional 0.1x TAEM (*e.g.*, "TAE/Mg^{2+}" had 1.1x TAEM buffer, "PBS" had 1x PBS + 0.1x TAEM). The salt concentrations for expanding poly(Am-*co*-S1dsA^{ac}R^{ac}1.154) hydrogel particles in varying buffers are listed in Supp. Table 3.

For poly(PEGDA10k-*co*-S1dsA^{ac}R^{ac}1.154) hydrogel triangle and particles, the hairpin stock solutions were at 200 μM per hairpin and were snap cooled in 1x TAEM. The hairpin concentration during hydrogel expansion was initially 20 μM per hairpin. The reaction volume for 10x buffers consisted of 10 μL of 10x buffer, 70 μL MilliQ water, and 10 μL each hairpin stock solution. The reaction volume for 5x buffers consisted of 20 μL of 5x buffer, 60 μL MilliQ water, and 10 μL each hairpin stock solution. Since the hairpins were snap cooled in 1x TAEM, the final buffer conditions in each condition included an additional 0.2x TAEM (*e.g.*, "TAE/Mg^{2+}" had 1.2x TAEM buffer, "PBS" had 1x PBS + 0.2x TAEM). The salt concentrations for expanding poly(PEGDA10k-*co*-S1dsA^{ac}R^{ac}1.154) hydrogel triangles and particles in varying buffers are listed in Supp. Table 4.

Images were captured every 30 minutes using a humidified Syngene G:Box EF2 gel imager equipped with a blue light transilluminator (Clare Chemical, Em. max ~450 nm) and a UV032 filter (Syngene, bandpass 572-630 nm) or on an Olympus IX73 fluorescence microscope. The equivalent Na^+ concentration in Supp. Tables 3 and 4 were calculated using equation 0 from Owczarzy *et al*.[6] The value of β was chosen to be 3.75, a value in the middle of the range of expected values for β.

$$[Na^+]_{eq} = [Monovalent\ Cations] + \beta\sqrt{[Divalent\ Cations]} \qquad (0)$$

Analysis of hydrogel triangle swelling

The relative change in the side length of the hydrogels was measured using MATLAB. The edge of the hydrogel was determined using standard intensity-based thresholding and mask image analysis. First, the intensity values of the image were globally adjusted using *imadjust* to saturate the bottom and top 1% of all pixel values. A Gaussian low-pass filter was applied to this adjusted image to reduce or remove background noise and generate the filtered image (FiltImg). The filtered image was then rotated so that the edges of the hydrogel triangle were not perfectly horizontal or vertical to aid in vertex detection.

A two-step process was used to determine the threshold used to find the hydrogel's edges. A general mask was generated from the filtered image using the following:

$$GenMask = FiltImg \geq 1.35 * mean(FiltImg) \quad (1)$$

The general mask (GenMask) is a logical matrix where values of one indicate the bulk hydrogel plus some extra background pixels. The threshold value was then calculated using equation 4.

$$PixOne\ matrix = FiltImg\ pixel\ values\ where\ GenMask\ pixels\ are\ 1 \quad (2)$$

$$PixZero\ matrix = FiltImg\ pixel\ values\ where\ GenMask\ pixels\ are\ 0 \quad (3)$$

$$Thresh = \frac{mean(PixOne)}{mean(PixZero) * \alpha} \quad (4)$$

The parameter alpha varied from image to image in order to provide a good agreement between the calculated boundary and the observed boundary of the hydrogel. The matrix PixZero generally represents the background pixels of the image. The final mask, with values of one indicating the pixels belonging to the hydrogel object (at least), was calculated using the threshold (Thresh) in equation 4:

$$HydMask = FiltImg \geq Thresh * mean(FiltImg) \quad (5)$$

Objects were removed (values set to 0) from HydMask if their total area was less than 700 pixels. The area of the hydrogels was at least 800 pixels in size. The boundary of the hydrogel was determined using MATLAB's *bwboundaries* function using a connectivity of 8.

The vertices of the hydrogel were determined from the extrema of the hydrogel object. The extrema and centroids of the objects in HydMask were determined using MATLAB's function *regionprops*. If background objects (*e.g.*, the side of the well) were found in HydMask, the object with a centroid closest to the center of the image was chosen to be the hydrogel object. *k*-means clustering was used to determine the location of the vertices from the 8 locations provided by the extrema of the hydrogel object. The algorithm was set to detect 4 clusters, and the 3 clusters that were the farthest apart were the vertices of the hydrogel. The average distance between these three clusters was used as the measure of the side length of the hydrogel. The relative change in the side length of the hydrogel was calculated using the measured side length (L) for each image in a time series relative to the side length prior to adding hairpins (L_0).

$$\frac{\Delta L}{L_0} = \frac{L - L_0}{L_0} \tag{6}$$

For each treatment variable plotted in the figures, the average relative change in side length ($\bar{x}$) was calculated by taking the mean value of at least three hydrogel swelling time series curves (*n* measurements). The 95% confidence interval bounds for each average measurement were calculated by calculating the standard deviation (*s*) of the swelling curves and multiplying by the 95th percentile of the Student's *t* distribution for *n*-1 degrees of freedom:

$$Bounds = \bar{x} \pm t \cdot \frac{s}{\sqrt{n}} \tag{7}$$

where *t* is calculated using MATLAB's *tinv* function.

Analysis of hydrogel particle swelling

Hydrogel particles were analyzed using MATLAB as previously described.[2] First, the area of the hydrogel particle was calculated from the number of pixels within the boundary of the particle in the micrograph. Next, the area (*A*) was converted into the radius (*r*) of the particle using:

$$r = \sqrt{A\pi} \tag{8}$$

The change in the radius, relative to the radius in the first image of the time series immediately after adding hairpins, was calculated using:

$$\frac{\Delta r}{r_0} = \frac{r - r_0}{r_0} \tag{9}$$

Supplementary Table 1: List of DNA sequences. Sequences were taken from previous literature[1,7] or designed using NUPACK.[4] Each hairpin's toehold lengths are listed as "primary toehold/secondary toehold" after the period in the strand name and in the role columns.

Strand Name	Role	Sequence
Crosslinks		
A_S1 (A1_a6)	Sequence set 1, 6bases primary toehold	/5Acryd/TAAGTT CGCTGTGGCACCTGCACG
R_S1 (R1_x3y3)	Sequence set 1, 3bases secondary toeholds	/5Acryd/CAA CGTGCAGGTGCCACAGCG TGG
A_S2 (A5_a10)	Sequence set 2 v1 & v2, 10bases primary toehold	/5Acryd/CTCTATCTAT CCATCACCCTCACCTTAC
A_S2a6 (A5_a6)	Sequence set 2 v3, 6bases primary toehold	/5Acryd/ATCTAT CCATCACCCTCACCTTAC
R_S2 (R5_x3y3)	Sequence set 2 v1 & v3, 3bases secondary toeholds	/5Acryd/GGT GTAAGGTGAGGGTGATGG TAA
R_S2x6 (R5_x6y6)	Sequence set 2 v2, 6bases secondary toeholds	/5Acryd/TGAGGT GTAAGGTGAGGGTGATGG TAAAGG
A_S3 (A2_a6)	Sequence set 3, 6bases primary toeholds	/5Acryd/CTGTCT GCCTACCACTCCGTTGCG
R_S3 (R2_x3y3)	Sequence set 3, 3bases secondary toeholds	/5Acryd/ATT CGCAACGGAGTGGTAGGC TTT
Hairpin Strands		
H1_S1_6/3 (H1_s1.6/3)	Hairpin monomer, sequence set 1, 6/3bases toeholds	CCA CGCTGTGGCACCTGCACG CACCCA CGTGCAGGTGCCACAGCG AACTTA
H2_S1_6/3 (H2_s1.6/3)	Hairpin monomer, sequence set 1, 6/3bases toeholds	TGGGTG CGTGCAGGTGCCACAGCG TAAGTT CGCTGTGGCACCTGCACG TTG
H1_S2_10/3 (H1_s5.10/3)	Hairpin monomer, sequence set 2, 10/3bases toeholds	TTA CCATCACCCTCACCTTAC TTGTAGATTG GTAAGGTGAGGGTGATGG ATAGATAGAG
H2_S2_10/3 (H2_s5.10/3)	Hairpin monomer, sequence set 2, 10/3bases toeholds	CAATCTACAA GTAAGGTGAGGGTGATGG CTCTATCTAT CCATCACCCTCACCTTAC ACC
H1_S2_8/3 (H1_s5.8/3)	Hairpin monomer, sequence set 2, 8/3bases toeholds	TTA CCATCACCCTCACCTTAC TTGTAGAT GTAAGGTGAGGGTGATGG ATAGATAG
H2_S2_8/3 (H2_s5.8/3)	Hairpin monomer, sequence set 2, 8/3bases toeholds	ATCTACAA GTAAGGTGAGGGTGATGG CTATCTAT CCATCACCCTCACCTTAC ACC
H1_S2_6/3 (H1_s5.6/3)	Hairpin monomer, sequence set 2, 6/3bases toeholds	TTA CCATCACCCTCACCTTAC TTGTAG GTAAGGTGAGGGTGATGG ATAGAT
H2_S2_6/3 (H2_s5.6/3)	Hairpin monomer, sequence set 2, 6/3bases toeholds	CTACAA GTAAGGTGAGGGTGATGG ATCTAT CCATCACCCTCACCTTAC ACC
H1_S2_8/6 (H1_s5.8/6)	Hairpin monomer, sequence set 2, 8/6bases toeholds	CCTTTA CCATCACCCTCACCTTAC TTGTAGAT GTAAGGTGAGGGTGATGG ATAGATAG
H2_S2_8/6 (H2_s5.8/6)	Hairpin monomer, sequence set 2, 8/6bases toeholds	ATCTACAA GTAAGGTGAGGGTGATGG CTATCTAT CCATCACCCTCACCTTAC ACCTCA
H1_S2_6/6 (H1_s5.6/6)	Hairpin monomer, sequence set 2, 6/6bases toeholds	CCTTTA CCATCACCCTCACCTTAC TTGTAG GTAAGGTGAGGGTGATGG ATAGAT
H2_S2_6/6 (H2_s5.6/6)	Hairpin monomer, sequence set 2, 6/6bases toeholds	CTACAA GTAAGGTGAGGGTGATGG ATCTAT CCATCACCCTCACCTTAC ACCTCA
H1_S3_6/3 (H1_s2.6/3)	Hairpin monomer, sequence set 3, 6/3bases toeholds	AAA GCCTACCACTCCGTTGCG GAACCT CGCAACGGAGTGGTAGGC AGACAG
H2_S3_6/3 (H2_s2.6/3)	Hairpin monomer, sequence set 3, 6/3bases toeholds	AGGTTC CGCAACGGAGTGGTAGGC CTGTCT GCCTACCACTCCGTTGCG AAT
H1_S1_ter (H1_s1.ter)	Terminator hairpin monomer, sequence set 1, 6/3bases toeholds	CCA CGCTGTGGCACCTGCACG TAGACT CGTGCAGGTGCCACAGCG AACTTA
H2_S1_ter (H2_s1.ter)	Terminator hairpin monomer, sequence set 1, 6/3bases toeholds	TGGGTG CGTGCAGGTGCCACAGCG GCCTAG CGCTGTGGCACCTGCACG TTG
H1_S2_mb	Hairpin monomer, sequence set 2, used in mass balance studies	TTACCATCACCCTCACCTTACTTGTAGATTTTTTGTAAGGTGA GGGTGATGGATAGATAGGGTAGGTGAATGGGA

H2_S2_mb	Hairpin monomer, sequence set 2, used in mass balance studies	TATGAGTGAGTTAGGATCTACAAGTAAGGTGAGGGTGATGG TTTTTCTATCTATCCATCACCCTCACCTTACACC
H1_S2_mb_ter	Terminator hairpin monomer, sequence set 2, used in mass balance studies	TTACCATCACCCTCACCTTACCTCTCCACTTTTTGTAAGGTGA GGGTGATGGATAGATAGGGTAGGTGAATGGGA
H2_S2_mb_ter	Terminator hairpin monomer, sequence set 4, used in mass balance studies	TATGAGTGAGTTAGGATCTACAAGTAAGGTGAGGGTGATGG TTTTTACGAGCCTCCATCACCCTCACCTTACACC
H1_S4_mb	Hairpin monomer, sequence set 4, used in mass balance studies	ATCCCACTCACACTCCACTCCCGCTCGCCTAATAGGAGTGGA GTGTGAGTGGAGTGGTAGGTTTAGGTGAGGTGG
H2_S4_mb	Hairpin monomer, sequence set 4, used in mass balance studies	GTTGTAAGTGAGAGTGGCGAGCGGGAGTGGAGTGTGAGT GGTAATACTACCACTCCACTCACACTCCACTCCACC
H1_S4_mb_ter	Terminator hairpin monomer, sequence set 4, used in mass balance studies	ATCCCACTCACACTCCACTCCGTGCTGGTTAATAGGAGTGG AGTGTGAGTGGAGTGGTAGGTTTAGGTGAGGTGG
H2_S4_mb_ter	Terminator hairpin monomer, sequence set 4, used in mass balance studies	GTTGTAAGTGAGAGTGGCGAGCGGGAGTGGAGTGTGAGT GGTAATAAAGGCGTCCCACTCACACTCCACTCCACC

Supplementary Table 2: List of buffers and their contents.

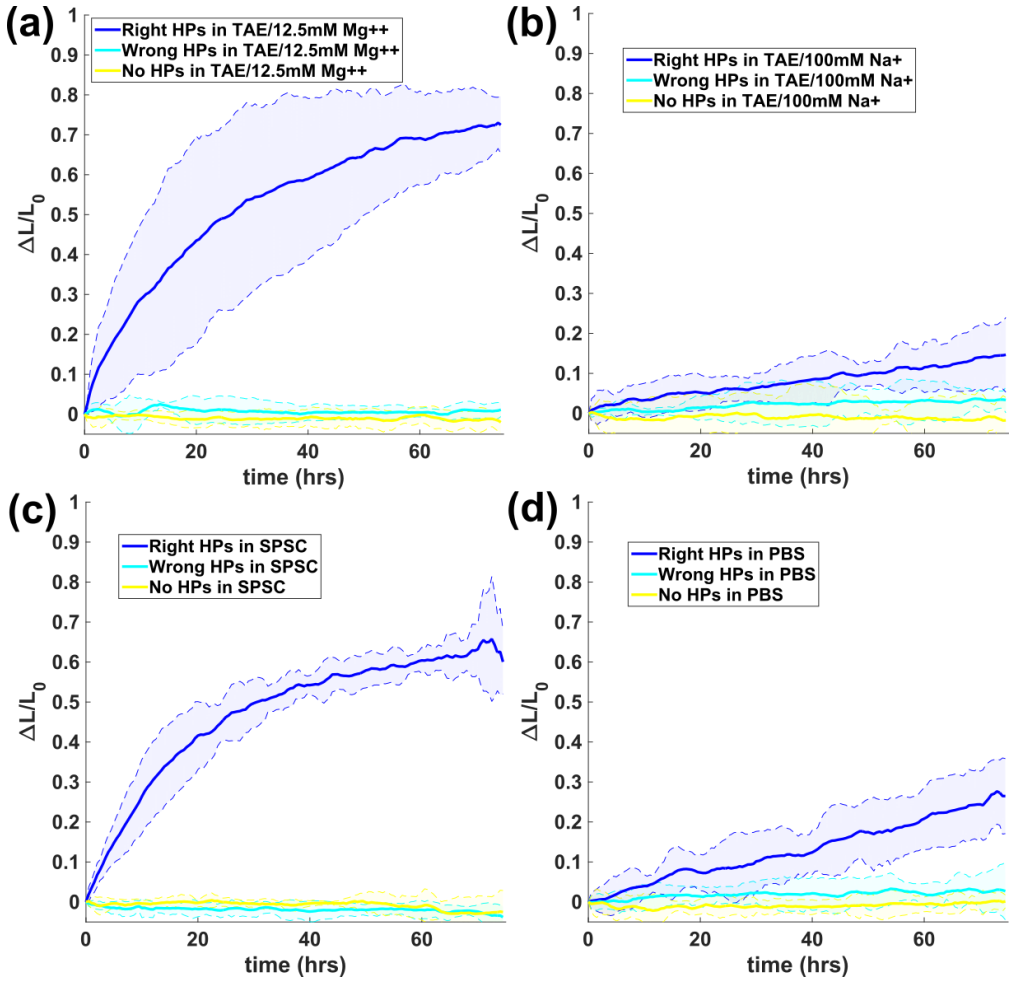
Buffer	Species
TAE/Mg^{2+}	40 mM Trizma, 1 mM EDTA, 20 mM Acetic Acid, 12.5 mM $Mg[acetate]_2$
TAE/Na^+	40 mM Trizma, 1 mM EDTA, 20 mM Acetic Acid, 100 mM NaCl
PBS	137 mM NaCl, 2.7 mM KCl, 10mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4
SPSC	1 M NaCl, 50 mM Na_2HPO_4, pH 8.0
TAE/Mg^{2+}/Na^+	40 mM Trizma, 1 mM EDTA, 20 mM Acetic Acid, 12.5 mM $Mg[acetate]_2$, 1 M NaCl

Supplementary Table 3: Calculating the equivalent Na^+ concentration for each buffer used for expanding poly(Am-*co*-S1dsA^{ac}R^{ac}1.154) hydrogel particles (Supp. Fig. 7). The value for β was 3.75 (Equation 0).[6] The calculation does not take pH into account. In this case, the hairpin stock solution accounts for 1/10th of the total volume in the well (400 μM per hairpin stock).

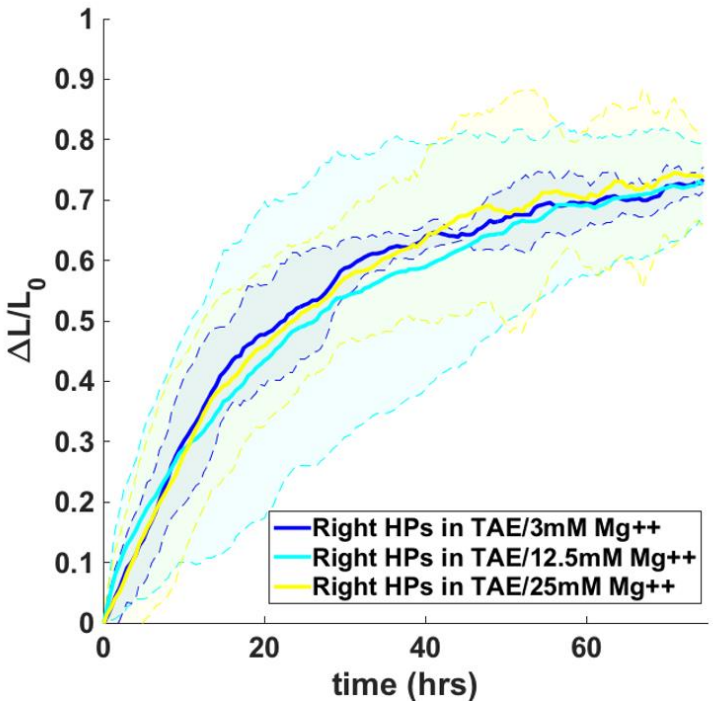
Buffer	$Tris^+$ (mM)	Na^+ (mM)	K^+ (mM)	Mg^{2+} (mM)	[$Mono^+$] (mM)	[Di^{2+}] (mM)	$[Na^+]_{eq}$ (mM)
TAE/Mg^{2+}	44	0	0	13.75	44	13.75	483.73
TAE/Na^+	44	100	0	1.25	144	1.25	276.58
PBS	4	157	4.5	1.25	165.5	1.25	298.08
SPSC	4	1100	0	1.25	1104	1.25	1236.58

Supplementary Table 4: Calculating the equivalent Na^+ concentration for each buffer used for expanding poly(PEGDA10k-*co*-S1dsA^{ac}R^{ac}1.154) hydrogel particles (Supp. Fig. 6). The value for β was 3.75 (Equation 0).[6] The calculation does not take pH into account. In this case, the hairpin stock solution accounts for 1/5th of the total volume in the well (200 μM per hairpin stock).

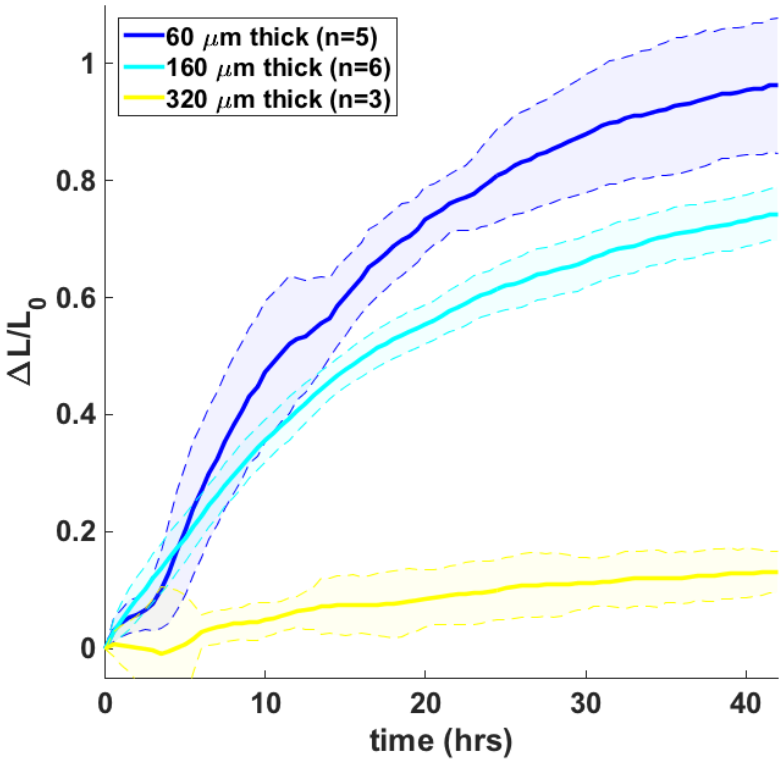
Buffer	$Tris^+$ (mM)	Na^+ (mM)	K^+ (mM)	Mg^{2+} (mM)	$[Mono^+]$ (mM)	$[Di^{2+}]$ (mM)	$[Na^+]_{eq}$ (mM)
TAE/Mg^{2+}	48	0	0	15	48	15	507.28
TAE/Na^+	48	100	0	2.5	148	2.5	335.50
PBS	8	157	4.5	2.5	169.5	2.5	357.00
SPSC	8	1100	0	2.5	1108	2.5	1295.50
TAE/Mg^{2+}/Na^+	48	1000	0	15	1048	15	1507.28



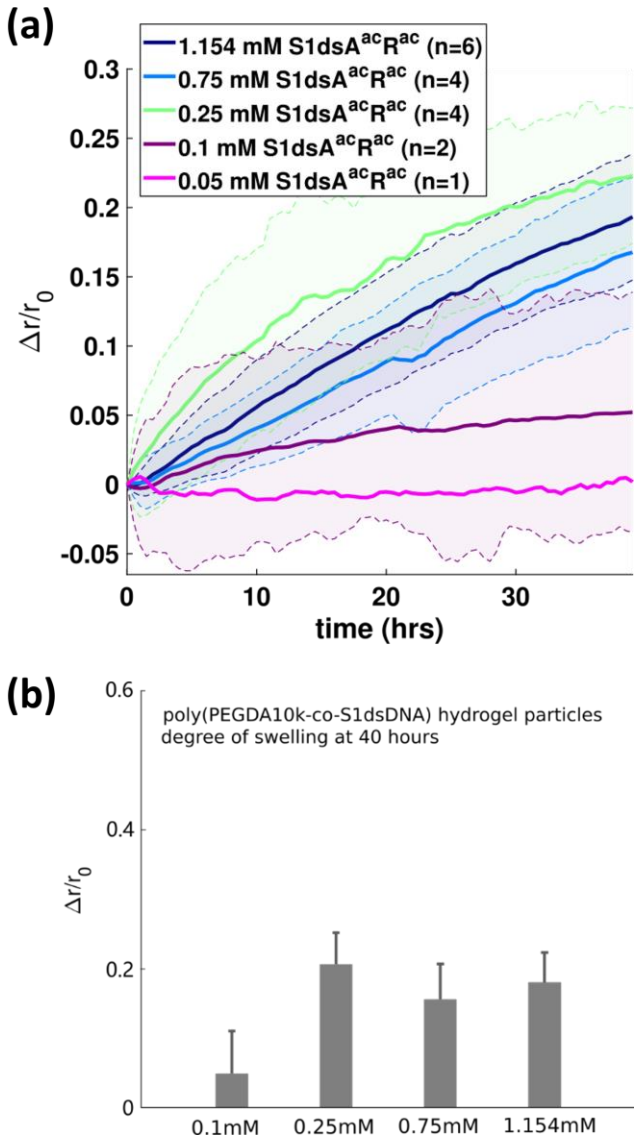
Supplementary Figure 1: The relative change in side length of poly(PEGDA10k-*co*-S1dsDNA1.154) hydrogels incubated with 20 μM per hairpin with S1 HPs (H1_S1_6/3 and H2_S1_6/3, Right HPs), S3 HPs (H1_S3_6/3 and H2_S3_6/3, Wrong HPs) and buffer only (no HPs) in (a) TAE/Mg^{2+}, (b) TAE/Na^{+}, (c) SPSC, (d) PBS. Solid curves are the averages of measurements of the number of hydrogels shown in the legend. Shaded regions show 95% confidence intervals as determined by standard deviations. See Supp. Table 2 for buffer contents.



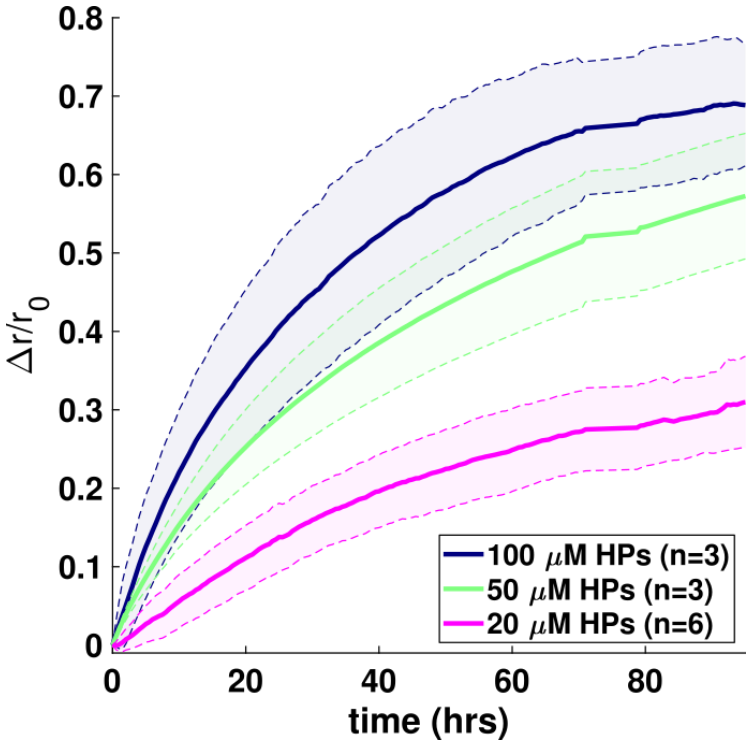
Supplementary Figure 2: The relative change in side length of poly(PEGDA10k-*co*-S1dsDNA1.154) hydrogel triangles incubated with 20 µM per hairpin with S1 HPs (H1_S1_6/3 and H2_S1_6/3) in 1xTAE buffer with 3 mM, 12.5 mM, or 25 mM Mg^{2+}. Solid curves are the averages of measurements of the number of hydrogels shown in the legend. Shaded regions show 95% confidence intervals as determined by standard deviations.



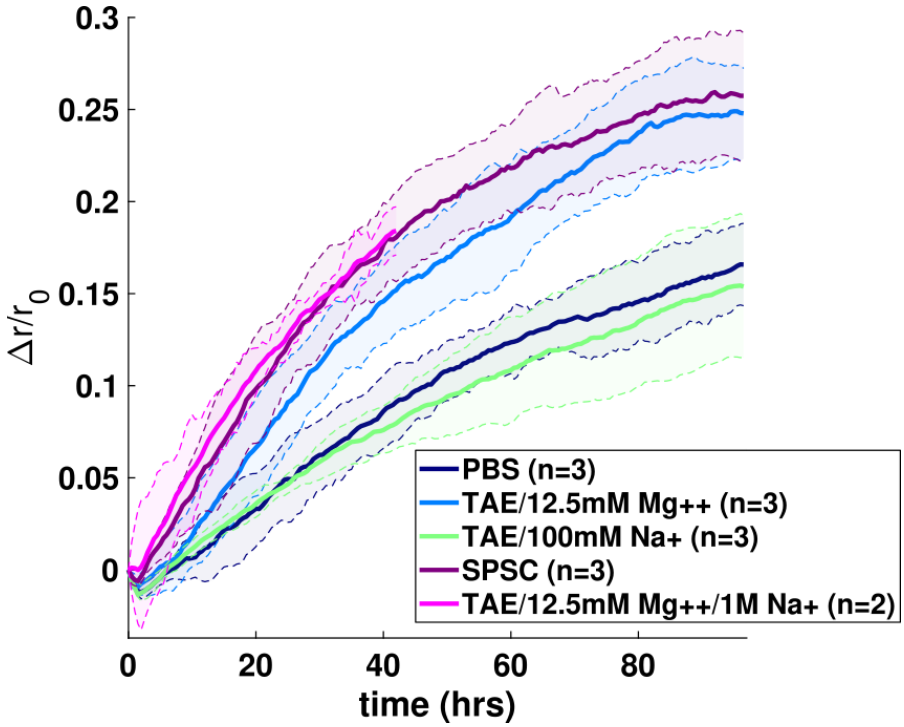
Supplementary Figure 3: Increasing the thickness of hydrogels reduces the extent of hydrogel expansion. The relative change in side length of poly(PEGDA10k-*co*-S1dsDNA1.154) hydrogels incubated with S1 HPs (H1_S1_6/3 and H2_S1_6/3) in 1x TAEM at 20 μM per hairpin type is shown. Solid curves are the averages of measurements of the number of hydrogels shown in the legend. Shaded regions show 95% confidence intervals as determined by standard deviations.



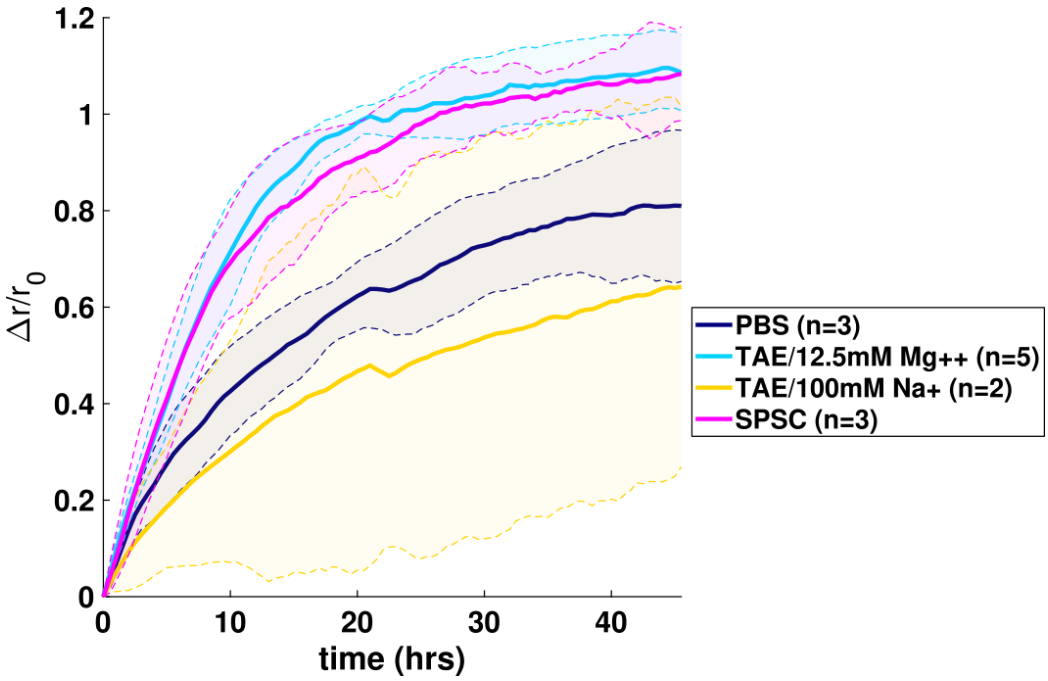
Supplementary Figure 4: (a) Relative change in average hydrogel radius of poly(PEGDA10k-*co*-S1dsDNA) hydrogel particles polymerized with varying concentrations of crosslinks. Sequence set 1 hairpin (H1_S1_6/3 and H2_S1_6/3) concentration was 20 µM per hairpin in all cases. The particles were expanded in 1x TAEM. Solid curves are the averages of measurements of the number of particles shown in the legend. Shaded regions show 95% confidence intervals as determined by standard deviations. (b) Bar graph of the degree of swelling at 40 hours. In this dataset involving gel particles, the degree of swelling across the samples is consistently low and there is a significant level of sample-to-sample variance, which complicates the interpretation of results and makes it challenging to draw definitive conclusions.



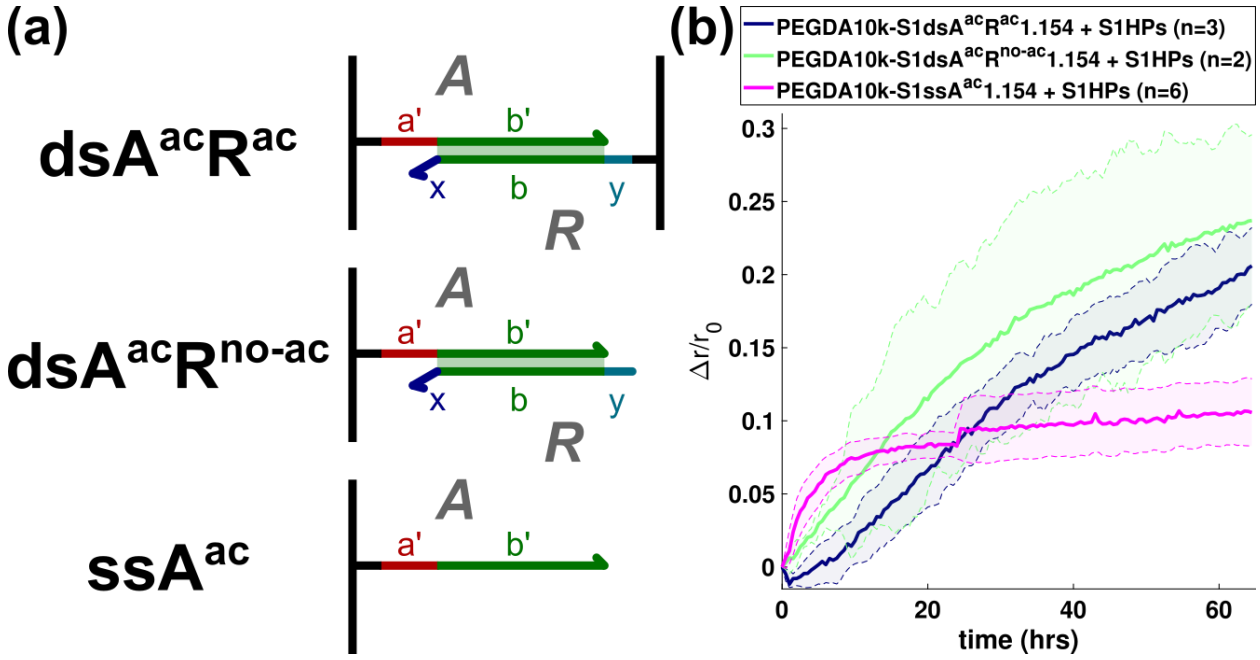
Supplementary Figure 5: Relative change in average hydrogel radius of poly(PEGDA10k-*co*-S1dsA^{ac}R^{ac}1.154) hydrogel particles incubated with varying concentrations of S1 HPs (H1_S1_6/3 and H2_S1_6/3). The particles were expanded in 1x TAEM. Solid curves are the averages of measurements of the number of particles shown in the legend. Shaded regions show 95% confidence intervals as determined by standard deviations.



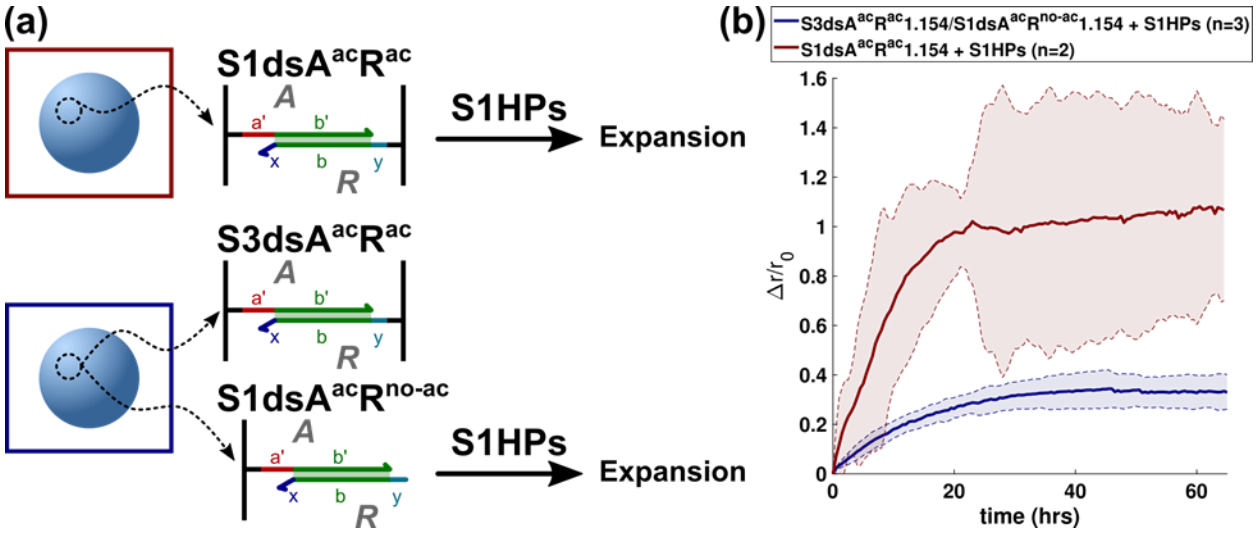
Supplementary Figure 6: Relative change in average hydrogel radius of poly(PEGDA10k-*co*-S1dsA^{ac}R^{ac}1.154) hydrogel particles incubated with 20 µM per hairpin (H1_S1_6/3 and H2_S1_6/3) in different buffer/salt conditions. Solid curves are the averages of measurements of the number of particles shown in the legend. Shaded regions show 95% confidence intervals as determined by standard deviations. See Supp. Tables 2 and 4 for buffer contents and calculated salt concentrations.



Supplementary Figure 7: Relative change in average hydrogel radius of poly(Am-*co*-S1dsA^{ac}R^{ac}1.154) hydrogel particles incubated with 20 µM per hairpin (H1_S1_6/3 and H2_S1_6/3) in different buffer/salt conditions. The fraction of hairpins that were terminator hairpins was 10%. Solid curves are the averages of measurements of the number of particles shown in the legend. Shaded regions show 95% confidence intervals as determined by standard deviations. See Supp. Tables 2 and 3 for buffer contents and calculated salt concentrations. We note that the experiment presented in Figure 4 also utilized 10% terminator hairpins and resulted in an earlier plateau time, which could be attributed to the differences in the polymer network used. The Am-DNA gels are crosslinked solely by modified DNA duplexes and do not have any additional covalent crosslinks, while the PEG-based gels possess a self-crosslinked polymer network that is limited by covalent C-C bonds and can attain swelling equilibrium even without the presence of terminator hairpins.



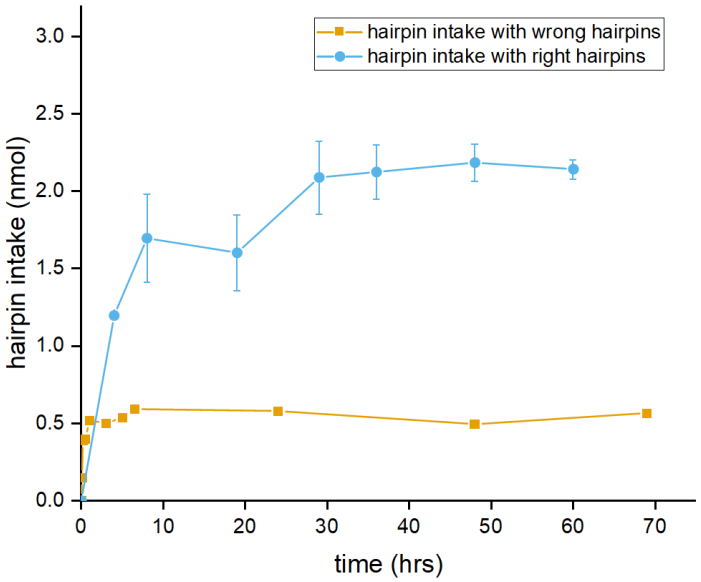
Supplementary Figure 8: Expanding poly(PEGDA10k-*co*-S1DNA1.154) particles with different forms of HCR initiators. (a) Methods of integrating DNA into hydrogels. Black lines indicate the polymer backbone. $dsA^{ac}R^{ac}$ indicates that both sides of the DNA duplex are anchored with the polymer backbone. $dsA^{ac}R^{no\text{-}ac}$ indicates that one side of the DNA duplex is anchored with the polymer backbone through A strand 5' end. ssA^{ac} indicates that only A strand (single strand DNA) is anchored with the polymer backbone through the 5' end. (b) Relative change in hydrogel radius of hydrogel particles polymerized with the different HCR initiators shown in (a). Hydrogels were expanded with 20 µM sequence set 1 hairpins (H1_S1_6/3 and H2_S1_6/3). The use of single-stranded HCR initiators($S1ssA^{ac}1.154$) increased the initial rate of expansion at the expense of a lower final degree of expansion. The particles were expanded in 1x TAEM. Solid curves are the averages of measurements of the number of particles shown in the legend. Shaded regions indicate 95% confidence intervals as determined by standard deviations.



Supplementary Figure 9: Expanding DNA-crosslinked polyacrylamide hydrogel particles poly(Am-*co*-DNA) using different methods of HCR initiation. (a) Schematic showing the DNA integrated into polyacrylamide particles with different HCR initiators. Upper gel: poly(Am-*co*-S1dsA^{ac}R^{ac}1.154) hydrogel particles. Lower gel: poly(Am-*co*-S3dsA^{ac}R^{ac}1.154- S1dsA^{ac}R$^{no\text{-}ac}$1.154) hydrogel particles. In the lower gel, S3dsA^{ac}R^{ac}1.154, inert to sequence set 1 HPs, was used to simulate the extra binding that S1dsA^{ac}R^{ac} created in the upper gel. (b) Relative change in hydrogel radius of poly(Am-*co*-DNA) hydrogel particles polymerized with the double-stranded DNA species shown in (a). The line color corresponds to the box around the hydrogel sphere in (a). Hydrogels were expanded using 20 µM sequence set 1 hairpins (H1_S1_6/3 and H2_S1_6/3), 10% of which were terminators. In the case of non-crosslinked dsDNA initiator-induced expansion, the hydrogel particles were crosslinked with a second set of DNA crosslinks set 3 with sequences that do not interact with sequence set 1. The particles were expanded in 1x TAEM. Solid curves are the averages of measurements of the number of particles shown in the legend. Shaded regions show 95% confidence intervals as determined by standard deviations.

Supplementary Text 1: Note on the Measurement of Hairpin Intake during DNA Polymerization.

To quantify the amount of hairpin intake during a gel swelling process, we first transferred the hairpin solution from the well to a tube at specific time points using a pipette. The total volume of the hairpin solution was then measured using the pipette to prevent errors caused by evaporation. Subsequently, the solution was diluted 100-fold with 1x TAEM, and the absorbance at 260 nm was measured. By referencing the absorbance values to a standard absorbance-concentration curve generated from known concentration hairpin mixtures and taking into account the total volume, we were able to calculate the overall quantity of hairpin present in the solution at a given time point. The hairpin intake was then determined by subtracting this calculated value from the initial amount of hairpins.



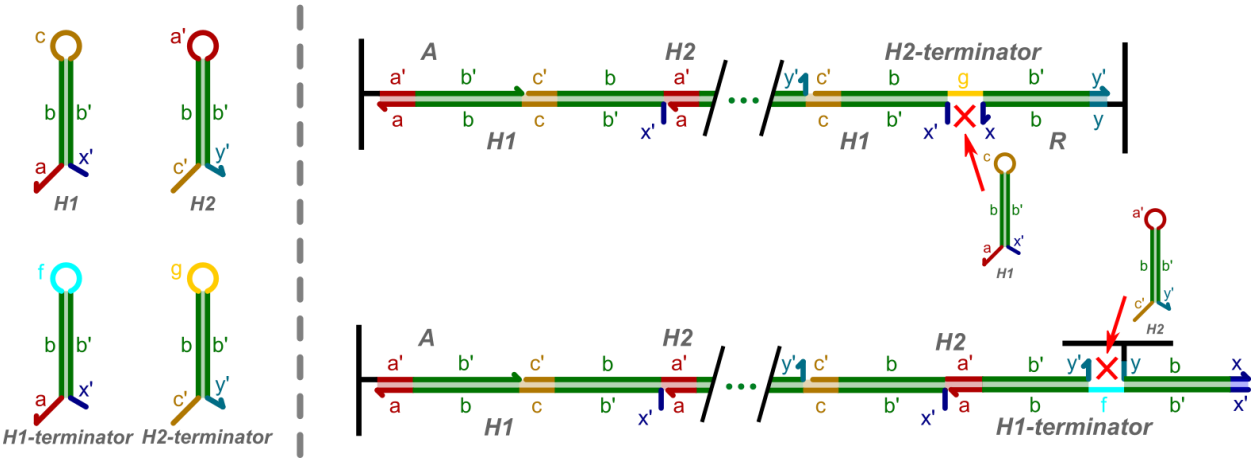
Supplementary Figure 10: The relative hairpin intake of poly(PEGDA10k-*co*-S2dsDNA1.154) hydrogel square (1mm side-length, 160 µm thickness) incubated with 60 µM per hairpin in 1xTAEM buffer. We note that this gel used is twice the volume as the triangle-shaped gels used in other studies of this paper and calculations in Supp. Text 1 have been adjusted accordingly. Right hairpins: S2 HPs (H1_S2_mb and H2_S2_mb, with 1% of H1_S2_mb_ter and H2_S2_mb_ter), which have the correct sequences that can direct gel swelling (n=3); wrong hairpins: S4 HPs (H1_S4_mb and H2_S4_mb, with 1% of H1_S4_mb_ter and H2_S4_mb_ter), which have the incorrect sequences and not inducing gel swelling (n=1). The intake with incorrect hairpins was used as a baseline for hairpin intake that did not participate in the HCR and was subtracted from the correct hairpin measurement in the above calculation.

We presume the amount of hairpin intake would vary depending on factors such as crosslink concentrations, hairpin design and concentrations, polymer type, etc., and the amount of hairpin intake for these varying parameters would be valuable for understanding mechanistic details. In

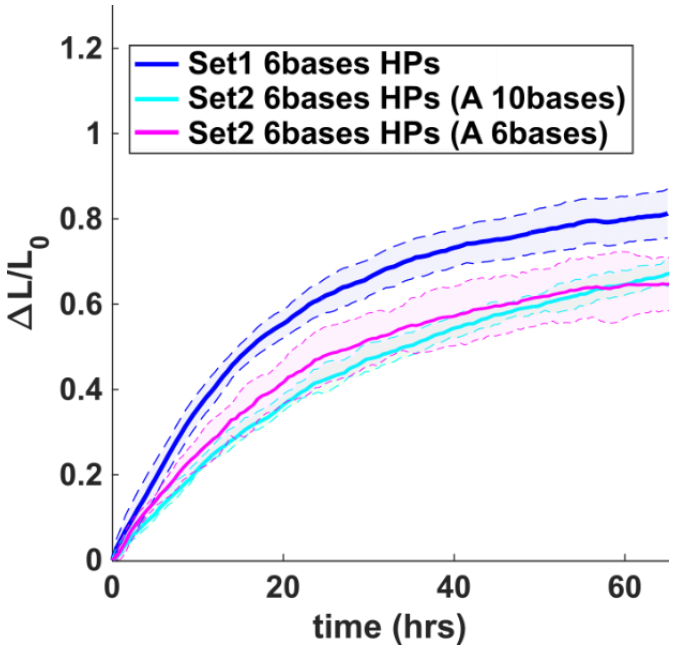
our specific experimental conditions, as shown in Supp. Fig. 10, we found that approximately 0.8 nmol of each type of DNA hairpin was consumed by the gel employed in this study, which had twice the volume of the triangle gels commonly used throughout this paper. Assuming a rough linearity between hairpin intake and crosslink amount, which is proportional to the gel volume, the triangle-shaped gels would exhibit an intake of 0.4 nmol for each type of hairpin. Initially, the DNA crosslinks were present at a concentration of 1.154 mM in the pre-gel solution. Previous studies have reported efficiency of DNA anchoring for acrydite-modified DNA in photopatterned PEG-based gels at approximately 55%.[8] The final DNA anchoring efficiency is therefore approximately 80% by simple calculation of probability as each DNA duplex contains two acrydite-modified groups. Therefore, the final concentration of DNA crosslinks inside the gel is about 0.92 mM. With a gel volume of 0.08 μL, the DNA crosslinks inside each gel amounted to 0.07 nmol. Consequently, the number of hairpins inserted for each crosslink point is approximately 12 (6 for each hairpin type).

Supplementary Text 2: Note on the function of terminator hairpins.

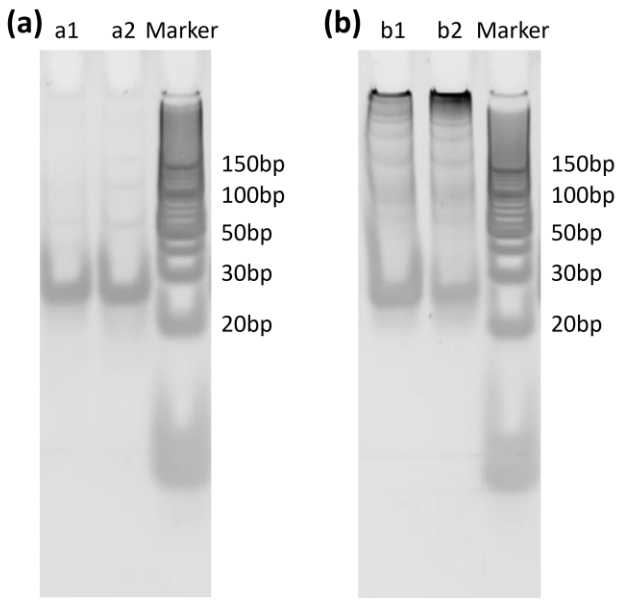
To regulate the final degree of swelling in DNA polymerization gels, a novel type of hairpin known as "terminator hairpins" was developed and utilized in conjunction with polymerizing hairpins. As described in [1], the loop domains (c and a') of the terminator hairpin were altered to contain non-complementary sequences. This modification ensures that when the terminator hairpin is inserted into the crosslink, no monomers can interact with the binding site, thereby providing control over the final degree of swelling.



Supplementary Figure 11: Schematic of the terminator hairpin creating sites where hairpins can not bind.



Supplementary Figure 12: Swelling comparison between set1 and set2 DNA polymerization gels. Dark blue: poly(PEGDA10k-*co*-S1dsDNA1.154) hydrogels incubated with S1 HPs (H1_S1_6/3 and H2_S1_6/3) in 1x TAEM at 20 µM per hairpin type. Light blue: poly(PEGDA10k-*co*-S2v1dsDNA1.154) hydrogels incubated with S2 HPs (H1_S2_6/3 and H2_S2_6/3) in 1x TAEM at 20 µM per hairpin type. Magenta: poly(PEGDA10k-*co*-S2v3dsDNA1.154) hydrogels incubated with S2 HPs (H1_S2_6/3 and H2_S2_6/3) in 1x TAEM at 20 µM per hairpin type. Solid curves are the averages of measurements of the number of hydrogels shown in the legend. Shaded regions show 95% confidence intervals as determined by standard deviations.



Supplementary Figure 13: Non-denaturing PAGE gel electrophoresis. Gels were made using 15% acrylamide/bis-acrylamide 19:1 solution in 1xTAEM and were polymerized with 0.5% by volume of 10% APS solution and 0.05% by volume TEMED. Each well contains 12µL of solution with a final concentration of 1 µM of each hairpin type. (a1) H1_S2_6/3 (51 bases); (a2) H2_S2_6/3 (51 bases); (b1) 0.05µM A_S2a6 + H1_S2_6/3 + H2_S2_6/3; (b2) 0.05 µM A_S2a6 + 0.05 µM R_S2 (A and R annealed) + H1_S2_6/3 + H2_S2_6/3. The solutions were prepared and allowed to sit for 3 days to mimic the gel swelling timescale before conducting gel electrophoresis. The gels were run in 1xTAEM buffer for 75 minutes at 150V and stained with 1x Sybr Gold for 15 minutes. The (a) gel shows that the great majority (>95%) of the hairpins remained in the single-strand form (the band observed between the 20 bp and 30 bp bands in the dsDNA ladder land). The faint bands observed traveling the same distance as the 50 bp bands in the dsDNA ladder correspond to the expected size of hairpin dimers but are very faint. The faintness of these bands with respect to the bands corresponding to hairpin monomers suggests that dimerization between hairpins is not a dominant effect driving swelling dynamics. The (b) gel shows that b2)

had a higher degree of polymerization, as indicated by the higher intensity near the loading area. There was no significant difference in bands near 50 bp, where the hairpins might form dimers. Together with the experimental findings in Figure 7, we can conclude that the presence of both A+R strands within the hydrogel is crucial to achieving a significant amount of swelling.

Supplementary Video 1: The swelling process of poly(PEGDA10k-*co*-dsDNA1.154) hydrogel triangles polymerized with sequence set 2 v3 DNA crosslinks using sequence set 2 hairpins in 1x TAEM (H1_S2_6/3 and H2_S2_6/3), the concentration of HPs was total 20 μM per hairpin.

Supplementary Video 2: The swelling process of poly(Am-*co*-DNA) gels hydrogel particles polymerized with sequence set 1 DNA crosslinks using sequence set 1 hairpins with 2% terminator strands in 1x TAEM (H1_S1_6/3 and H2_S1_6/3, H1_S1_ter and H2_S1_ter), the concentration of HPs was total 20 μM per hairpin.

Supplementary Video 3: The swelling process of poly(Am-*co*-DNA) gels hydrogel particles polymerized with sequence set 1 DNA crosslinks using sequence set 1 hairpins with 10% terminator strands in 1x TAEM (H1_S1_6/3 and H2_S1_6/3, H1_S1_ter and H2_S1_ter), the concentration of HPs was total 20 μM per hairpin.

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