Supplementary Information: An autonomous polymerization motor powered by DNA hybridization

[1]Suvir Venkataraman, [1]Robert M. Dirks, [2,3]Paul W.K. Rothemund, [2,3]Erik Winfree and [1,4,*]Niles A. Pierce

[1]Department of Bioengineering, [2]Department of Computer Science, [3] Department of Computation & Neural Systems, [4]Department of Applied & Computational Mathematics
California Institute of Technology, Pasadena, CA, 91125, USA, *niles@caltech.edu

Methods

Native gel electrophoresis

20 μM stock solutions of each oligomer were prepared in ultrapure water (resistance of 18 MΩ-cm). All reactions were performed in 1× SPSC buffer (1 M NaCl, 50 mM Na_2HPO_4; pH 7.5). The following solutions were prepared by diluting aliquots of stock solutions with ultrapure water and 5× SPSC buffer: 12 μM H1, 12 μM H2 and 1.2 μM A·R_L. Both hairpins were snap cooled (heat at 95°C for 2 minutes, incubate on ice for 1 minute then leave at room temperature for 30 minutes) and the A·R mixture was annealed (heat at 95°C for 2 minutes, slow cool at 1°C / min to room temperature) prior to use. For each lane the sample was prepared by mixing 1 μL of solution(s) for each each species and diluting up to 12 μL with 1× SPSC buffer, thus resulting in reaction concentrations relative to 1 μM. The samples were incubated overnight at room temperature. 3 μL of 50% glycerol solution was added to every sample prior to running in a 2% native agarose gel in 1× LB buffer [Faster Better Media LLC] at 150 Volts for 50 minutes. The reference lane is a 100 bp DNA ladder [New England BioLabs (#N3231L)]. The gel was imaged using a Fuji FLA-5100 fluorescent scanner, by exciting with a 532 nm laser and passing the emission through a 570 ±10 nm bandpass filter. To image non-fluorescently labelled DNA the gel was post-stained using SYBR Gold [Molecular Probes] and then scanned again, this time the sample was excited with a 473 nm laser and the emission was passed through a 530 ± 10 nm bandpass filter.

For the time-course gel of Figure S1, reactions were prepared as above and the A·R_L complex was added the specified amount of time prior to mixing with 3 μL of 50% glycerol solution and being run on a 20% native polyacrylamide gel in 1× TBE buffer (90 mM Tris-borate, 2 mM EDTA; pH ≈ 8.3) for 12 hours at 300V and 15°C. The ladder sample was supplemented with SYBR Gold stain in order to visualise it on the fluorescent scan. The gel was scanned using the fluorescent scanner by exciting with a 532 nm laser and passing the emission through a 570 ± 10 nm bandpass filter.

Fluorescence capture

A 2 μM solution of T·A_{ext}·R_L was prepared by diluting 20 μM stock solutions with 5× SPSC buffer and ultrapure water, and annealing. For both the control reaction and the run, 10 pmol

of this complex were diluted up to 500 μL in 1× SPSC buffer and transferred to 700 μL quartz cuvettes [Hellma]. Fluorescence data were obtained using a fluorometer from Photon Technology International with the temperature set to 23°C. The fluorescence of the two samples was measured (Baseline) with the excitation and emission wavelengths set to 550 nm and 563 nm, respectively, and all slits set to a bandpass of 4 nm. The two solutions were transferred back into microcentrifuge tubes and mixed with 25 μL of streptavidin coated beads [Pierce #53117] that had been rinsed twice in 1× SPSC buffer. The mixtures were wrapped in aluminium foil and incubated for 24 hours at 4°C on a rotator. The unbound DNA was then eluted by filtering the mixtures through 0.45 μm centrifugal filter units [Millipore #UFC30HV0S], the eluates were transferred into cuvettes and their fluoresence was measured (Eluate 1). The beads, and bound DNA, were recovered from the filters by resuspending in buffer and transferred to microcentrifuge tubes. They were then centrifuged at 1300 ×g for 4 minutes and the supernatant was removed.

The following solutions were prepared by diluting stock solutions with 5× SPSC buffer and ultrapure water: 6.6 μM A·R (annealed) , 10 μM H1 and 10 μM H2 (snap-cooled). For the control reaction, 990 pmol of the A·R complex were diluted up to 500 μL in 1× SPSC buffer. For the run 990 pmol of the A·R complex were mixed with 1 nanomole of each hairpin and diluted up to 500 μL in 1× SPSC buffer. These solutions were mixed with the beads and bound DNA. The samples were incubated for 30 minutes at room temperature on a rotator. Unbound DNA was eluted and its fluorescence was measured as before (Eluate 2). The beads, and bound DNA, were recovered from the filters as before.

A 10 μM reaction solution of T* was prepared by diluting stock solution with 5× SPSC buffer and ultrapure water and snap cooling. For both the control reaction and the run, 20 pmol of T* were diluted up to 500 μL in 1× SPSC buffer. These solutions were mixed with the beads and bound DNA. The samples were incubated overnight at room temperature on a rotator. The released DNA was eluted and its fluorescence was measured as before (Eluate 3).

For each eluate, forty readings of the fluorescence were recorded over the course of twenty minutes, immediately after it was collected, in the same mannner as described above for Baseline. The reading for 1× SPSC buffer was 55 counts/sec.

Fluorescence stepping

All reactions were performed in 3.5 mL quartz cuvettes [Hellma], in 1× SPSC buffer. The cuvettes were always sealed using parafilm in order to minimize evaporation. The following solutions were prepared by diluting 20 μM stock solutions of each species with 5× SPSC buffer and ultrapure water: 0.6 μM A·R_{2L}, 16 μM H1, 16 μM H2, 16 μM $H1_Q$, 16 μM $H2_Q$. Aliquots of solutions of A·R were annealed and the hairpins were snap cooled. For all three samples: run (Sample 1), the dilution control (Sample 2), and the quenching control (Sample 3), 60 pmol of A·R_{2L} were added to 2675 μL 1× SPSC buffer. 600 pmol of H1 and H2 were added to samples 1 and 2. 600 pmol of $H1_Q$ and $H2_Q$ were added to Sample 3. The samples were incubated overnight at room temperature in the dark.

Fluorescence data were obtained using a fluorometer from Photon Technology International with the temperature set to 23°C. Excitation and emission wavelengths were 491 nm and 517 nm respectively for FAM, and 592 nm and 608 nm respectively for Texas Red. All slits were set to a bandpass of 4 nm. Readings were taken for 20 minutes at each step of the reaction.

After the baseline fluorescence values (Reading 1) were recorded for samples 1 and 2, another 600 pmol of $H1_Q$ and $H2_Q$ were added to samples 1 and 3. 600 pmol of H1 and H2 were also added to sample 2 at this point. All samples were incubated for 24 hours, at room temperature in the dark. For this step (Reading 2), fluorescence measurements were recorded for samples 1 and 3. Another

600 pmol of H1 and H2 were then added to samples 1 and 2. The samples were incubated for another 24 hours, at room temperature in the dark. The final fluorescence measurements (Reading 3) were then recorded for samples 1 and 2.

Atomic force microscopy

Aliquots of 5′ biotin labelled A and 3′ biotin labelled R solutions were prepared by mixing 1 μL of each 2 μM stock solution with 1 μL of 5× SPSC buffer and 2 μL of ultrapure water, then annealing. 1 μL of 20 μM H1 and H2 were each supplemented with 1 μL of 5× SPSC buffer and 3 μL of ultrapure water and snap cooled. The hairpin solutions were mixed with the A·R solution and 185 μL of 1× SPSC buffer. This mixture was incubated overnight at room temperature. The sample was imaged in liquid phase using an atomic force microscope [Veeco - Nanoscope] in tapping mode. 5 μL of the sample was deposited on a freshly cleaved mica surface. The sample was then rinsed five times with 200 μL of 1× TAE/Mg^{2+} buffer (40mM Tris, 19mM acetic acid, 1mM EDTA, 12.5mM magnesium acetate; pH 8.0). 5 μL of 0.2 μM streptavidin was then added and incubated for five minutes. The sample was again rinsed five times with 200 μL of 1× TAE/Mg^{2+} buffer. Immediately prior to imaging, the sample buffer was supplemented with 30 mM Ni^{2+} to improve visualisation of duplex DNA.

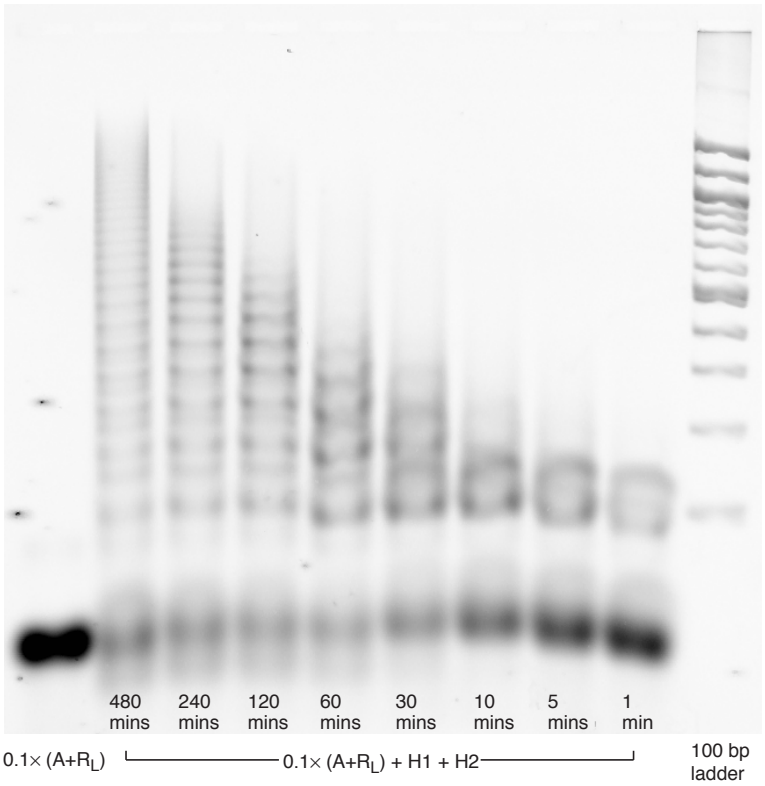
Prepartion of DNA origami rectangles

DNA origami rectangles were formed by folding a 7,249-nucleotide single-stranded DNA scaffold (M13mp18, New England Biolabs #N4040S) using 192 short 'staple' oligonucleotides (pp. 42-45 supplementary materials part 1) [1]. The two long sides of the rectangles each contain 16 single stranded 'tether' regions with distinct sequences, to which other DNA complexes can be attached via hybridization [2]. Two types of rectangles were created: R rectangles and A rectangles. The staple mixture used to fold the origami was modified for each type of rectangle to display the appropriate letter on the surface of the origami as a pattern of protruding hairpins. By prepending the A strand sequence to the reverse complements of eight of the tether regions, eight A strands were attached to A rectangles:

Origami.A_1: 5′/GCACGTCCACGGTGTCGCTTGAAT TGAAAGTATTAAGAGGCTATTATTCTGAAACA/3′
Origami.A_2: 5′/GCACGTCCACGGTGTCGCTTGAAT AGACAAAAGGGCGACAGGTTTACCAGCGCCAA/3′
Origami.A_3: 5′/GCACGTCCACGGTGTCGCTTGAAT AACGTCAAAAATGAAAAAACGATTTTTTGTTT/3′
Origami.A_4: 5′/GCACGTCCACGGTGTCGCTTGAAT GCTTATCCGGTATTCTAAATCAGATATAGAAG/3′
Origami.A_5: 5′/GCACGTCCACGGTGTCGCTTGAAT CATAGGTCTGAGAGACGTGAATTTATCAAAAT/3′
Origami.A_6: 5′/GCACGTCCACGGTGTCGCTTGAAT GAAGATGATGAAACAAAATTACCTGAGCAAAA/3′
Origami.A_7: 5′/GCACGTCCACGGTGTCGCTTGAAT GCCGTCAATAGATAATCAACTAATAGATTAGA/3′
Origami.A_8: 5′/GCACGTCCACGGTGTCGCTTGAAT CAGGAACGGTACGCCATTAAAGGGATTTTAGA/3′

By appending the Rickettsia strand sequence to the reverse complements of eight of the tether regions (separated by a 6-T spacer), eight R strands were attached to R rectangles:

Origami.R_1: 5′/TGAAAGTATTAAGAGGCTATTATTCTGAAACA TTTTTTGGTGCGACACCGTGGACGTGCAAC/3′
Origami.R_2: 5′/AGACAAAAGGGCGACAGGTTTACCAGCGCCAA TTTTTTGGTGCGACACCGTGGACGTGCAAC/3′
Origami.R_3: 5′/AACGTCAAAAATGAAAAAACGATTTTTTGTTT TTTTTTGGTGCGACACCGTGGACGTGCAAC/3′
Origami.R_4: 5′/GCTTATCCGGTATTCTAAATCAGATATAGAAG TTTTTTGGTGCGACACCGTGGACGTGCAAC/3′
Origami.R_5: 5′/CATAGGTCTGAGAGACGTGAATTTATCAAAAT TTTTTTGGTGCGACACCGTGGACGTGCAAC/3′
Origami.R_6: 5′/GAAGATGATGAAACAAAATTACCTGAGCAAAA TTTTTTGGTGCGACACCGTGGACGTGCAAC/3′
Origami.R_7: 5′/GCCGTCAATAGATAATCAACTAATAGATTAGA TTTTTTGGTGCGACACCGTGGACGTGCAAC/3′
Origami.R_8: 5′/CAGGAACGGTACGCCATTAAAGGGATTTTAGA TTTTTTGGTGCGACACCGTGGACGTGCAAC/3′



Supplementary Figure S1: Fluorescent scan of a native polyacrylamide time-course gel (labeled strand R_L). The formation of each base pair represents a displacement of approximately 0.34 nm along the helix axis for B form DNA [3]. Comparison with the 100 bp ladder suggests that the average rate of polymerization decreases with time and is on the order of nanometers per minute.

The origami were prepared by mixing 5 μL 10× TAE/Mg^{2+} buffer (1×: 40mM Tris, 19mM acetic acid, 1mM EDTA, 12.5mM magnesium acetate; pH 8.0), 5 μL of M13 DNA [0.01 μM], 5 μL of the relevant staple mixture [150 μM total], 3.2 μL of Origami.A_{1-8} [10 μM total] or Origami.R_{1-8} [10 μM total], 1.6 μL 3′ biotin labelled R [20 μM] and 30.2 μL ultrapure water and annealing. 16 μM solutions of H1 and H2 were prepared by diluting stock solutions with 10× TAE/Mg^{2+} buffer and ultrapure water and snap cooled. 64 pmol of snap cooled H1 and H2 were added to 10 μL of the origami, which results in approximately a 10:1 ratio of hairpins to polymerisation sites, and incubated for three hours at room temperature. Samples were imaged in liquid phase, in tapping mode as above. After deposition on the mica surface, the samples were rinsed with 1× TAE/Mg^{2+} buffer and supplemented with 30 mM Ni^{2+} prior to imaging.

References

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