Supporting Information

Rewritable memory by controllable nanopatterning of DNA

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Materials and methods

Synthetic Oligonucleotides and Preparation of the Memory Device. Initial oligonucleotide sequence design was performed using automated sequence design software to optimize the probability of adopting various combinations of target secondary structures[1]. Further manual optimization to minimize the formation of unfavorable secondary structure was performed using the secondary structure prediction and visualization capabilities of mfold 3.0[2]. All DNA oligonucleotides were purchased from Integrated DNA Technologies, Inc. DNA labelling with fluorophore or quencher and subsequent purification by HPLC or PAGE depending on the base length were done by the supplier and the resulting oligomers were used without further purification. DNA stock solutions (20~50 μM) were prepared in TE buffer (pH 8.0) and their concentrations were determined by molecular extinction coefficient at 260 nm supplied by the manufacturer. Due to substantial errors in the concentration determined by optical density, actual relative concentrations among the complementary oligonucleotides were determined by PAGE analysis in which relative amounts were varied and gel band intensities for residual unhybridized oligonucleotide were compared. The memory device in the all 'off' 000 state (final concentration = 2 μM) was prepared by adding D strands sequentially from D5 to D1 into a modified PBS buffer (MPBS, 50 mM sodium phosphate, 1 M NaCl, pH 6.5). Each strand addition was followed by 30 min incubation at 37 ^{o}C, followed by one final incubation for 1 hour at 37 ^{o}C.

Non-denaturing PAGE Analysis. All the reactions for non-denaturing PAGE were performed in the same MPBS buffer. The typical reaction volume for PAGE experiments was 5 μl. Writing or erasing processes were performed by mixing corresponding W or E strands (5 pmol) with the device (5 pmol) in MPBS, followed by 1 hour incubation at 37 ^{o}C. The whole sample was loaded in 8 % non-denaturing gel. Gel electrophoresis was accomplished in 1× TBE buffer at 50 V and 20 ^{o}C, and the bands were visualized by silver staining.

Fluorescent Analysis. Fluorescence measurements were carried out with a fluorometer equipped with a photomultiplier detector (PTI Co.). Unless otherwise specified, both bandwidths for excitation and emission were set to 4 nm and the working volume for measurements was 100 μl. All the reactions were performed in the MPBS buffer. The devices with one 'on' address were prepared by separately mixing three fluorescent 000 devices (5 pmol) with the corresponding W strand (5.5 pmol) in 5 μl of MPBS. After 1 hour incubation at 37 ^{o}C, the mixture was diluted 20-fold with 95 μl of MPBS and then the emission spectrum of each fluorescent device was recorded. For the cycling experiment (Figure 4*B*), W or E was added in slight excess over the device at each stage to ensure that each reaction reached completion. The whole cycle was performed as follows; 1. add W1 (1.1 eq.) and W2 (1.1 eq.) to state 000 for toggle to state 110; 2. add W3 (1.1 eq.) and E1 (1.2 eq.) for toggle to 011; 3. add W1 (1.3 eq.) and E2 (1.2 eq.) for toggle to 101; 4. add E1 (1.4 eq.) and E3 (1.2 eq.) to return to state 000. Each addition was followed by 1 hour incubation at 37 ^{o}C.

Ferguson analysis of device species

We performed a Ferguson analysis of the four device species from Figure 3A (D1+D2, D1+D2+D3, D1+D2+D3+D4, D1+D2+D3+D4+D5) as well as for linear duplex markers ranging from 100 to 1000 bp at 100 bp increments. PAGE experiments were performed at 5%, 6.7%, 8.7% and 10.7% acrylamide. Figure S1 shows Ferguson plots for the four device species and four duplex markers (100 bp, 200 bp, 300 bp, 400 bp) based on absolute mobility. Table S1 lists the retardation coefficient for each Ferguson plot (corresponding to

the negative of the slope), which increases with the effective molecular radius of the species[3]. Evidently, single-stranded overhangs dramatically increase the effective molecular radius of a duplex backbone. Varying the concentration of the full device (D1+D2+D3+D4+D5) does not affect gel mobility or cause band splitting (see Figure S3). These results are consistent with monomeric device assembly using a single copy of each D strand, as opposed to dimeric or trimeric assembly involving multiple copies of each strand.

Kinetic Analysis of the Writing and Erasing Processes

The kinetic trace for writing exhibits single exponential behavior under pseudo-first order reaction conditions (Figure 3*E*), so under these conditions, the writing process is thought to follow simple first-order kinetics:

$$\begin{aligned} \frac{d[M]}{dt} &= -k_w[W]_0[M] \\ \frac{d[MW]}{dt} &= k_w[W]_0[M] \end{aligned} \quad . \qquad [\mathbf{1}]$$

Here, $[MW]$ is the concentration of memory device in the new state, k_w is a rate constant for the writing process, $[W]_0$ is the initial concentration of W strand, and $[M]$ is the concentration of memory device in the initial state. Integration of Eq. **1** yields single exponential functions for $[M]$ and $[MW]$ whose exponents are linearly dependent on $[W]_0$:

$$\begin{aligned} [M] &= [M]_0 e^{-k_w[W]_o t} \\ [MW] &= [M]_0(1 - e^{-k_w[W]_o t}) \end{aligned} \qquad [\mathbf{2}]$$

Because both M and MW contribute to fluorescence intensity, the total intensity with respect to time is

$$f(t) = \varepsilon_{MW}[M]_0 + [M]_0(\varepsilon_M - \varepsilon_{MW}) \cdot e^{-k_w[W]_o t}, \qquad [\mathbf{3}]$$

where ε is the molar fluorescence intensity. Accounting for the measurement delay resulting from mixing by pipetting, t_p, the actual fluorescence is

$$f(t) = \varepsilon_{MW}[M]_0 + [M]_0(\varepsilon_M - \varepsilon_{MW})e^{-k_w[W]_o(t+t_p)}. \qquad [\mathbf{4}]$$

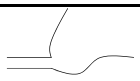
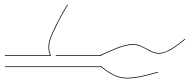
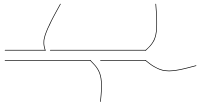
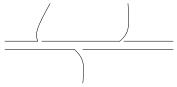
Despite uncertainties in ε and t_p, it is possible to obtain $k_w[W]_0$ by fitting Eq. **4** to kinetic traces. Figure S4*A* shows typical fitting results for the writing process. Consistent with Eq. **4**, the observed rate constants are linearly proportional to $[W]_0$. The k_w values are obtained by linear regression as illustrated in Figure S4*A*.

The erasing process showed a double-exponential kinetic trace under pseudo-first order reaction conditions (Figure 3*E*). The observed rate constant for each phase was obtained by curve fitting to a double exponential function. Figure S4*B* shows typical fitting results. Differing dependence on $[E]_0$ concentration suggests that the rate determining step is bimolecular for the fast phase and unimolecular for the slow phase. The rate constants for the fast phase are obtained by a linear regression and those for the slow phase are taken to be the mean value.

References

1. Dirks, R. M. & Pierce, N. A. (2003) *J. Comput. Chem.* **24**, 1664-1677.
2. Zuker, M. (2003) *Nucleic Acids Res.* **31**, 1-10.
3. Rodbard, D. & Chrambach, A. (1971) *Anal. Biochem*. **40**, 95-134.

Table 1. Comparison of retardation coefficients from the Ferguson plot of Figure S1.

DNA species	Retardation coefficient	Structure
100 bp marker	0.078	
200 bp marker	0.085	
300 bp marker	0.092	
400 bp marker	0.100	
D1+D2	0.101	
D1+D2+D3	0.128	
D1+D2+D3+D4	0.141	
D1+D2+D3+D4+D5	0.145	

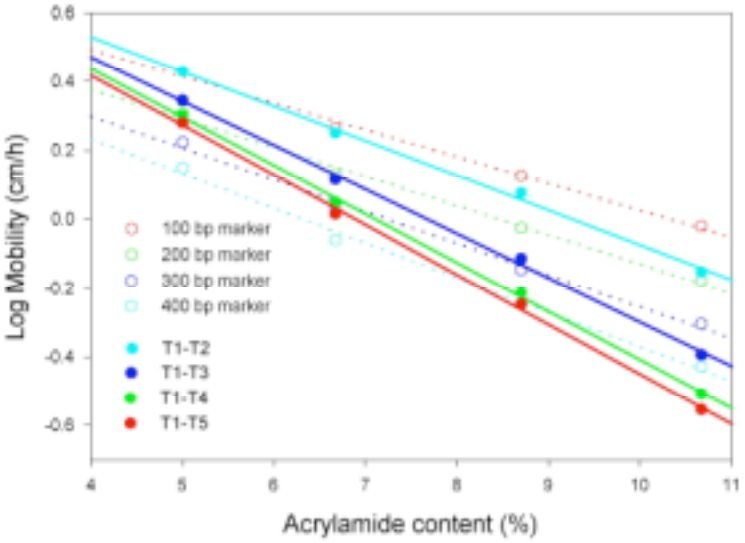


Figure S1: Ferguson plots of size markers and four device species. Lines represent least squares fits to the data.

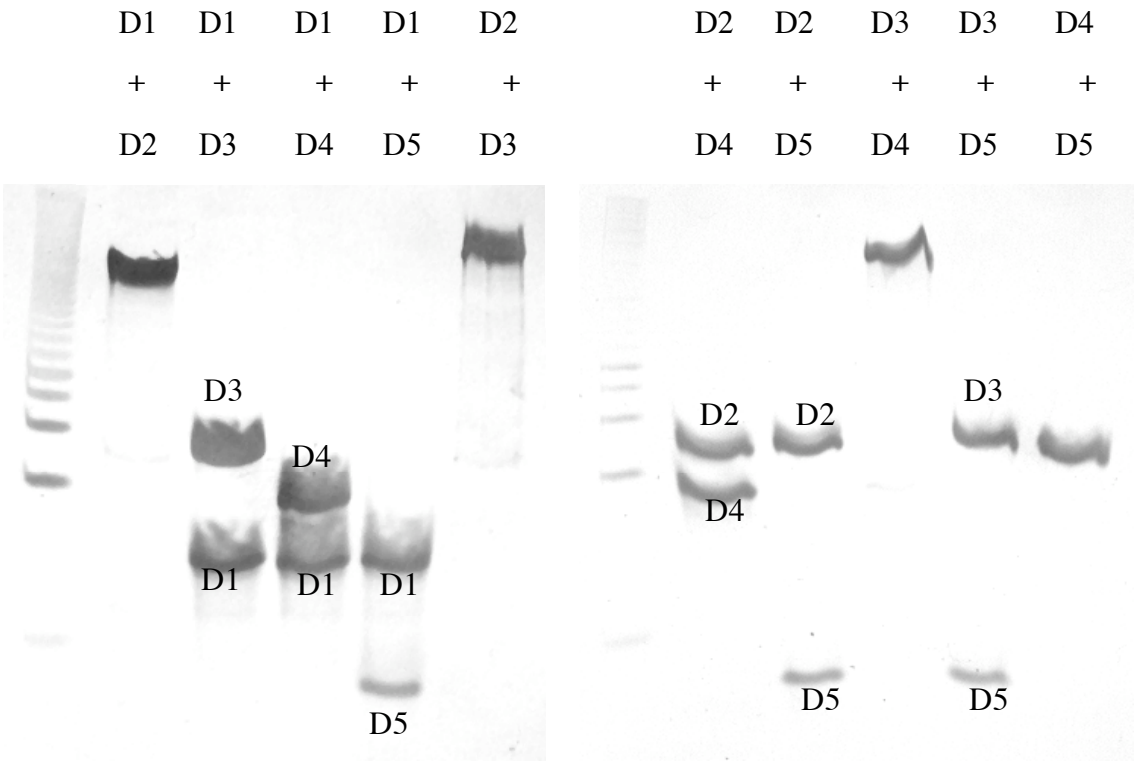


Figure S2: Formation of hybridization complexes between D strands. Two out of five D strands (each 15 pmol) were mixed in 5 μl of MPBS and allowed to stand at 37 ^{o}C for 30 min. The reaction mixtures were loaded in native PAGE (15 %).

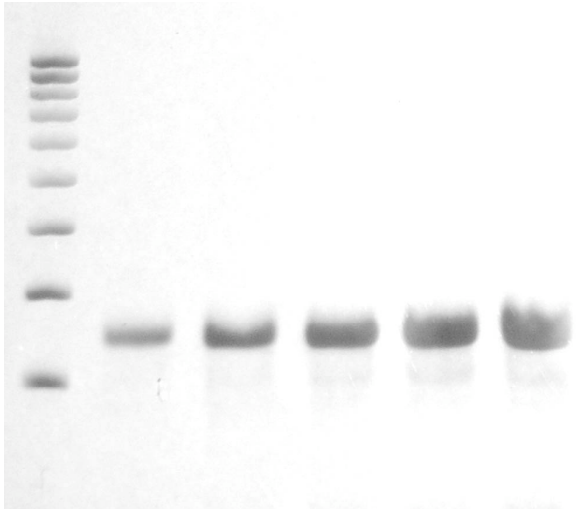


Figure S3: Non-denaturing PAGE (5%) with varying concentration of memory device (D1+D2+D3+D4+D5). 10 μl of sample was loaded in each lane. Lane 1, duplex size markers; 2, 0.5 μM device; 3, 1.0 μM device; 4, 1.5 μM device; 5, 2.0 μM device; 6, 2.5 μM device.

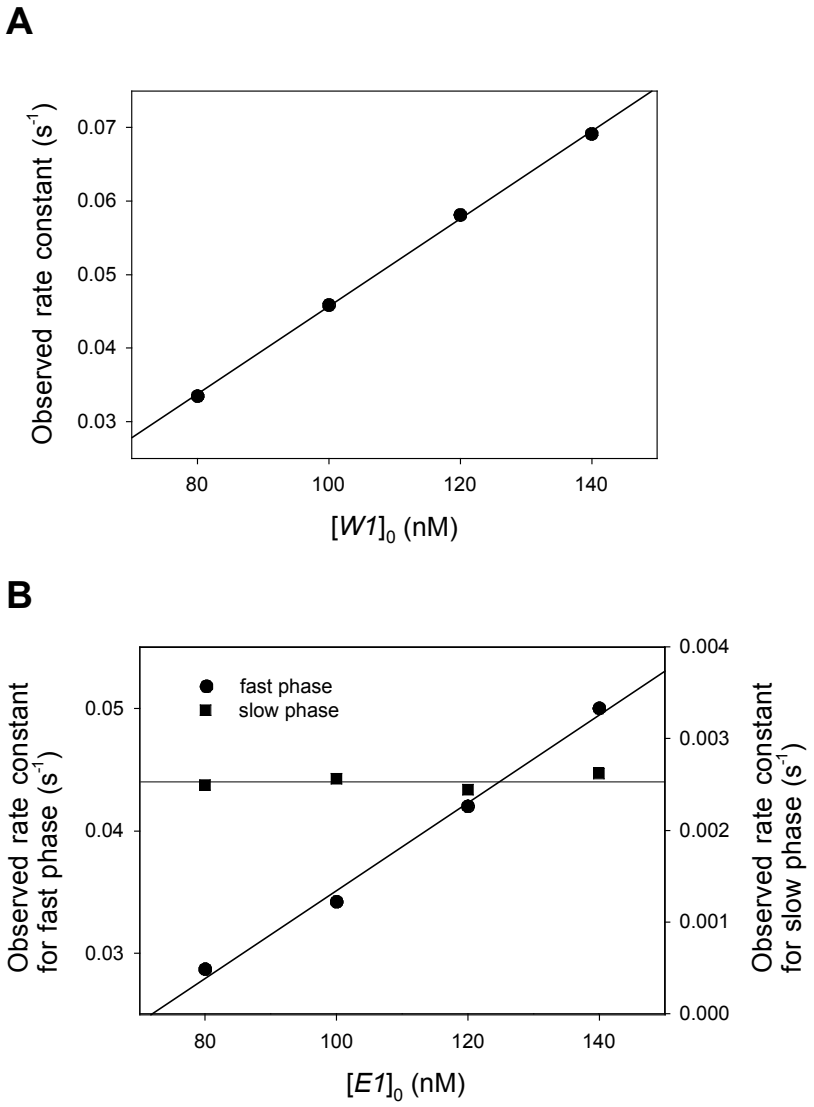


Figure S4: Concentration dependence of observed rate constants for writing and erasing processes at address 1. Kinetic measurements were performed under pseudo-first order reaction conditions employing excess W1 (BHQ1-labelled) or E1 strand (80 ~ 120 nM) and a fluorescent memory device (Hex-labelled at 5´ end of D3, 10 nM) in 100 µl of MPBS. (*A*) Observed rate constants for the writing process: 000 to 100. (*B*) Observed rate constants for the erasing process: 100 to 000.