Nature Methods

High-throughput single-molecule optofluidic analysis

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Supplementary Figure 1	Schematics of Microfluidic Mixing Device
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Note: Supplementary Software is available on the Nature Methods website.

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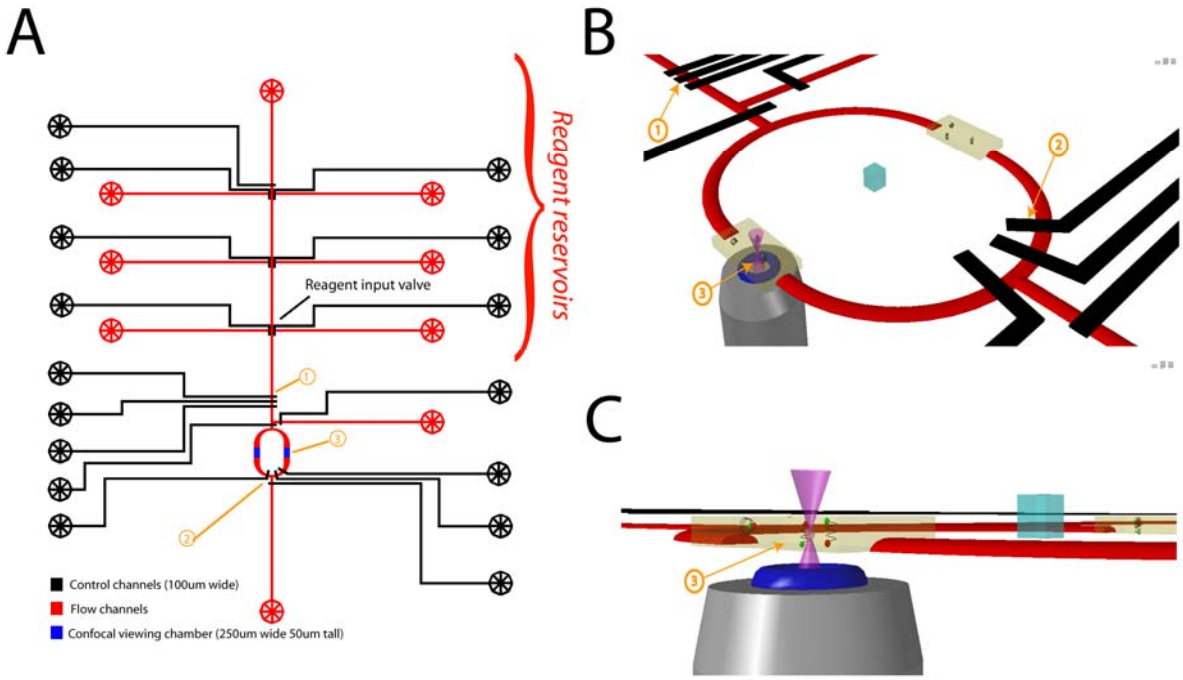
SUPPLEMENTAL FIGURES



SUPPLEMENTARY FIGURE 1

SUPPLEMENTARY FIGURE 1. Schematic of the two-layer microfluidic mixing device. Control channels are shown in black and flow channels are shown in red. Locations of the peristaltic injection pump ('1'), mixing pump ('2'), and observation chamber ('3') are indicated with arrows. (**a**) Top view of full device. Seven independently addressable reagent inputs are labeled. (**b,c**) Three-dimensional rendering of mixing ring. Within the observation chamber the confocal detection volume is represented as inverted cones emanating from a microscope objective lens located beneath the chip. Molecules are fed into and mixed into the mixing ring prior to acquisition. Elements of the microfluidic device are to scale, however the objective is not for schematic purposes. The blue cube in the middle serves as a scale bar with 100 um edges.

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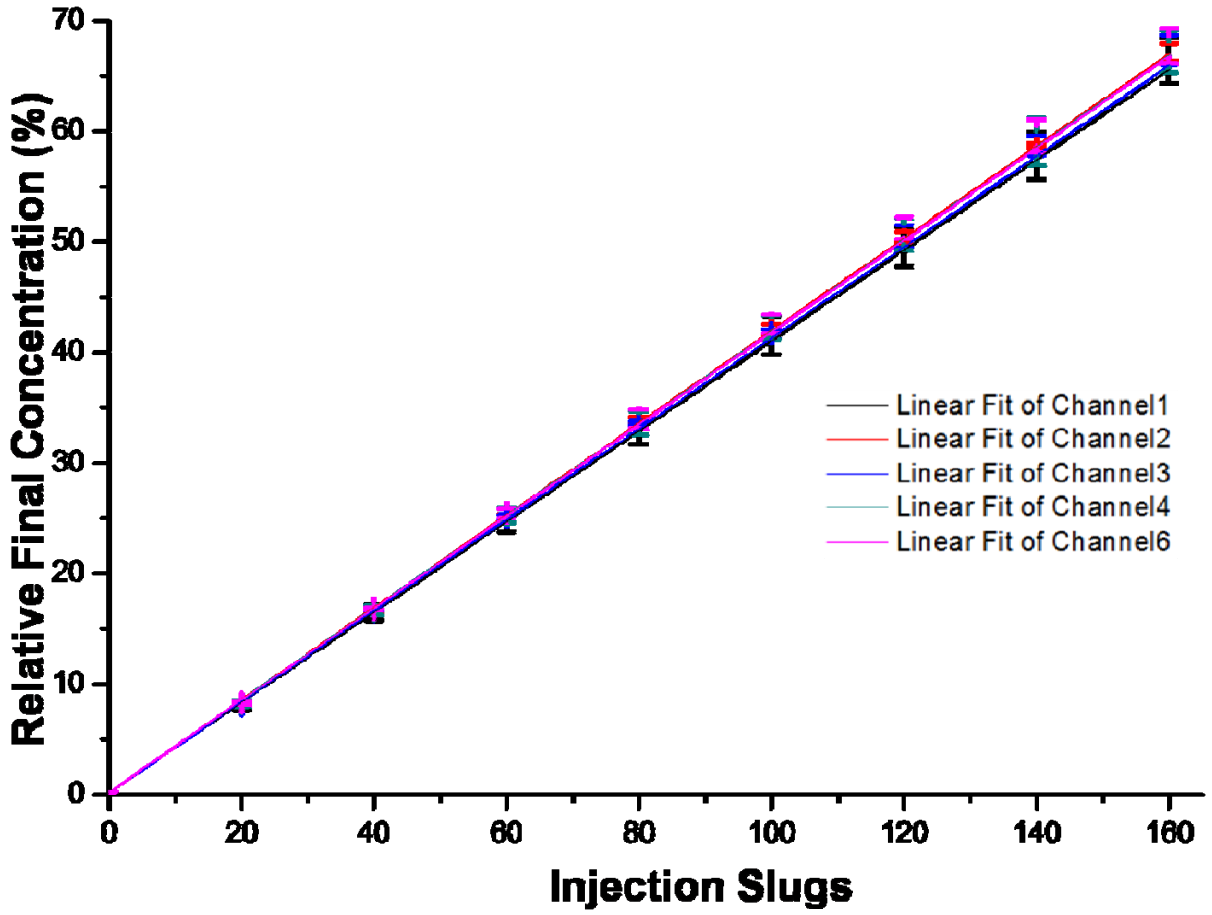

SUPPLEMENTARY FIGURE 2.

SUPPLEMENTARY FIGURE 2. Calibration of injection pump. “Injection slug” refers to a single cycle of the peristaltic pump, the minimum injection increment. Calibration was conducted with the fluorescent dye Alexa Fluor 488, and relative concentration was determined by normalizing measured fluorescence intensity after mixing to the fluorescence intensity measured in a saturated ring. The linear relationship between injection slugs and relative concentration is used to determine reagent concentrations in ring. Injection from six input channels are calibrated independently

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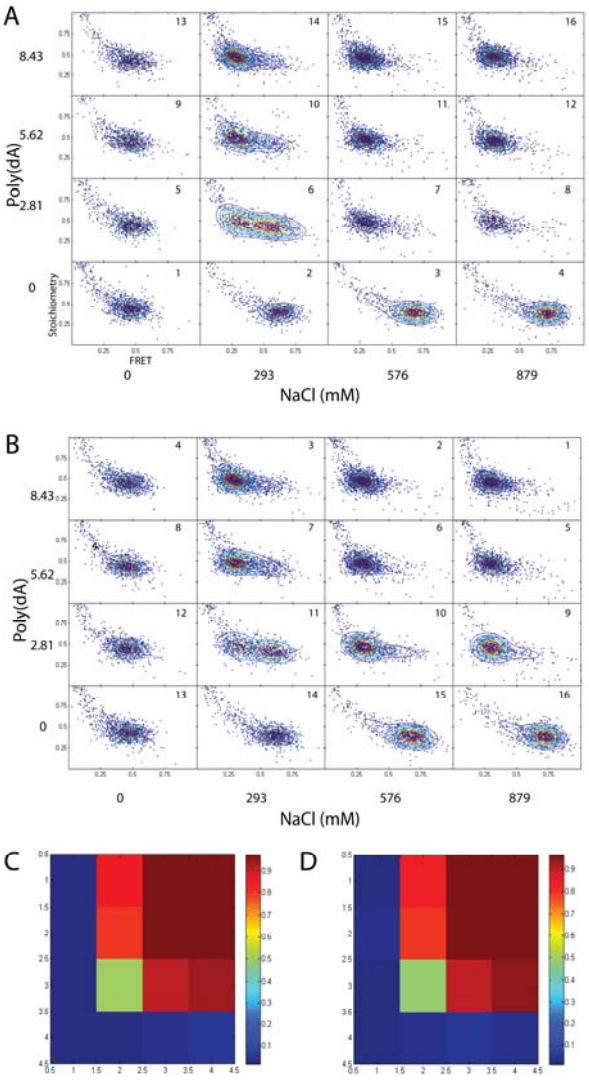

SUPPLEMENTARY FIGURE 3.

SUPPLEMENTARY FIGURE 3. Changing the sequence of injection of samples into the mixing ring did not result in a noticeable change in hybridization ratio. This demonstrates that the reproducibility of the microfluidic device is orthogonal to the order of titrations. This is shown using identical experiments carried out in forward (**a**) and reverse (**b**) order of fluid mixing with corresponding heatmaps of hybridization (**c**) and (**d**), respectively. Experiment carried out in forward and reverse order titrations of 2 M NaCl and 45 nM 10 dA on doubly labeled 20 dT DNA were done in increasing concentrations and repeated in reverse order. The order of the each acquisition sequence is indicated in the top right of each E-S histogram.

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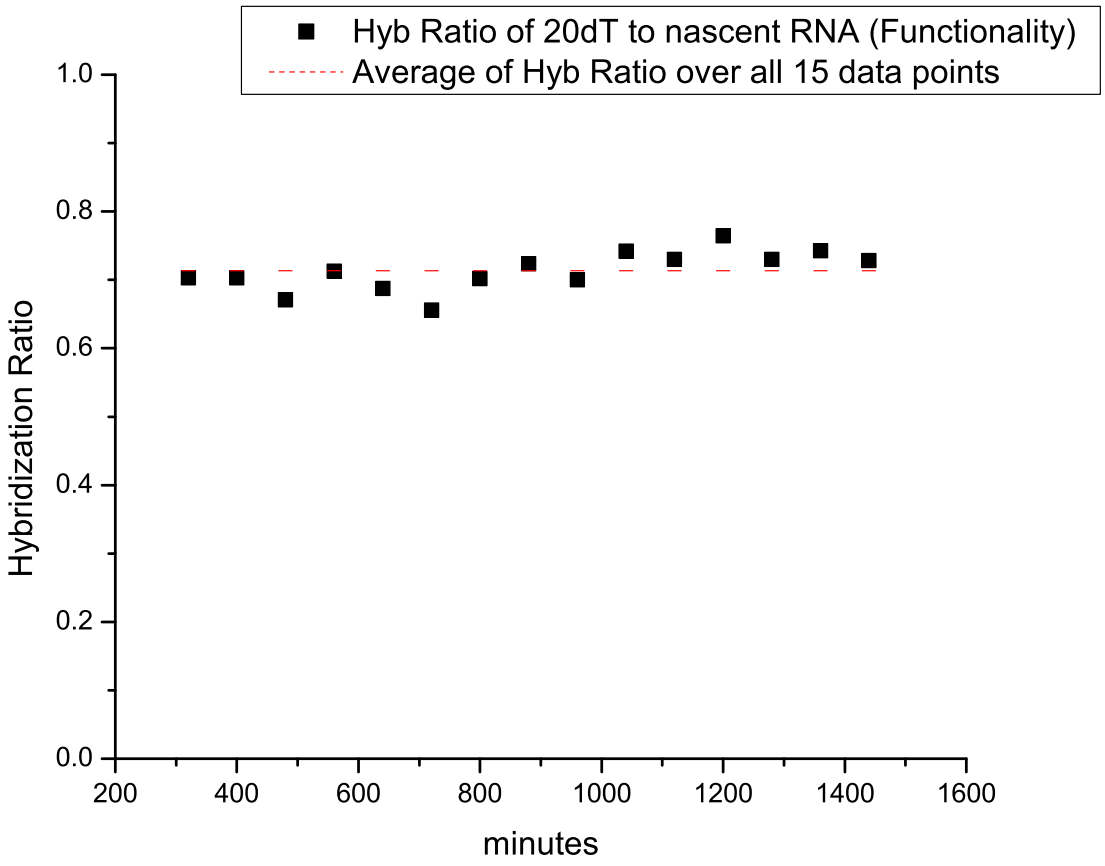

SUPPLEMENTARY FIGURE 4.

SUPPLEMENTARY FIGURE 4. Sample integrity does not change over the timescale of microfluidic experiments. 227.6 pM of RNA polymerase open complex was diluted into the ring with 250 pM of probe DNA to transcribe under 40 mM of KGlu. This transcription reaction was repeated 15 times in a period of 1120 minutes (320 minutes after the RNAP open complex assembly, when measurement would typically begin, to 1440 minutes) with identical conditions. Throughout the 24 hour period, the transcription efficiency remains constant within a standard deviation of 4%. This demonstrates that the protein/DNA complex and the dyes are stable throughout the course of the experiment, and that the experimental results are highly reproducible.

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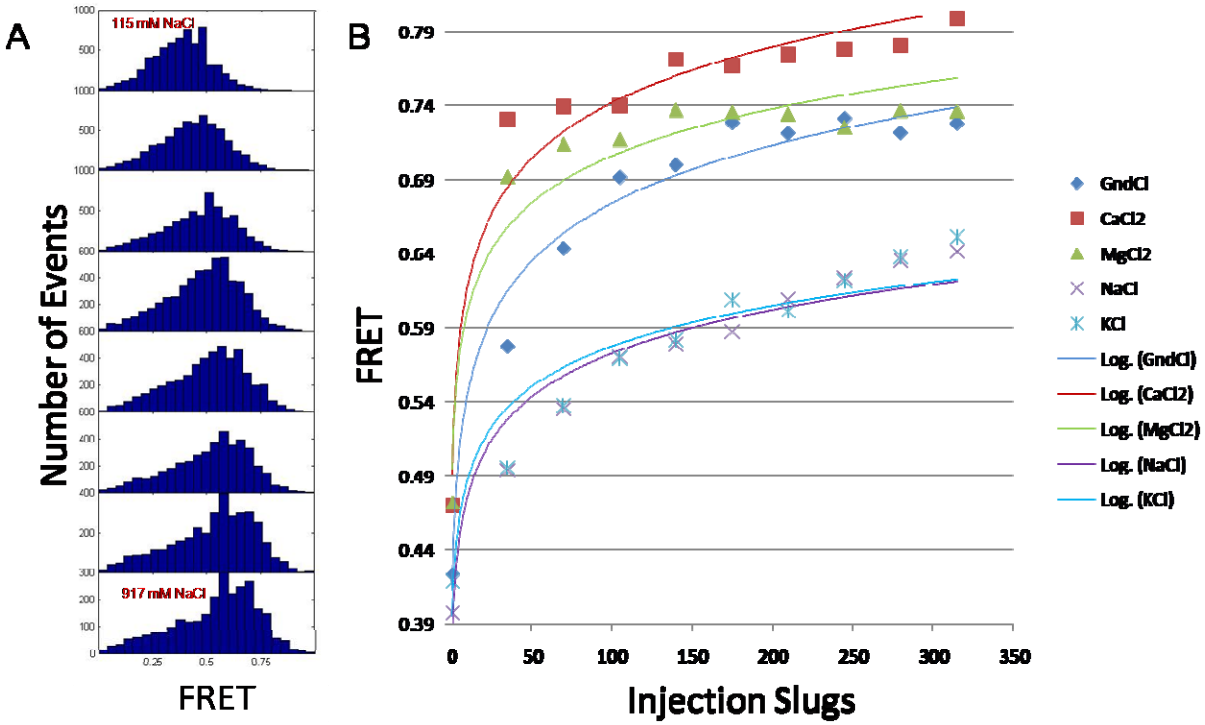

SUPPLEMENTARY FIGURE 5.

SUPPLEMENTARY FIGURE 5. Effect of ionic strength on ssDNA (**a**) Increase in ionic strength collapses ssDNA, as reported by FRET histograms (plotted as function of [NaCl]; same data as in bottom row of Figure 1C). (**b**) FRET change as function of salt concentration for 5 different salts (Guanidine HCl, $CaCl_2$, $MGCl_2$, NaCl, and KCl; derived from data as in (a)) Distributions were fitted to Gaussians (assuming normal distributions), peak positions are plotted as a function of salt concentration (injection slugs) to demonstrate the effect of ionic strength, divalent ions, and Hofmeister salts on ssDNA conformation.

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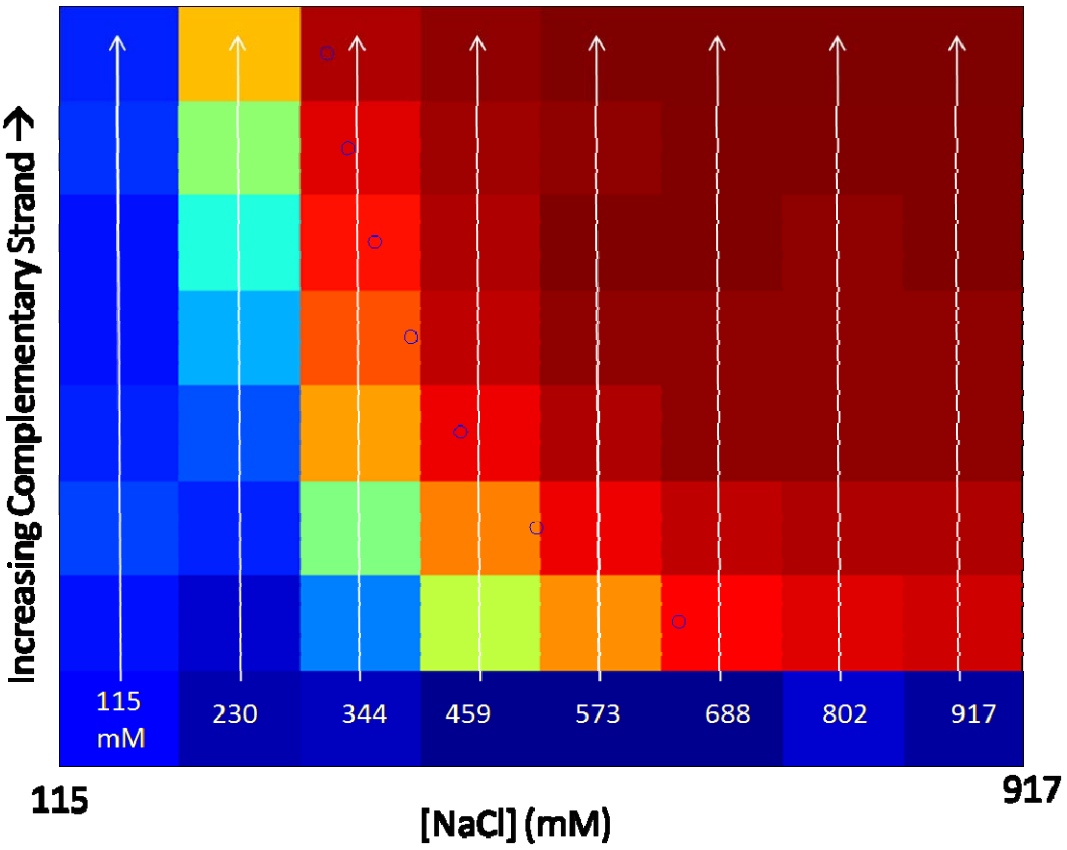

SUPPLEMENTARY FIGURE 6.

SUPPLEMENTARY FIGURE 6. Here, we show Fig. 1D, annotated with theoretical hybridization midpoints (blue circles for each row of NaCl titration). Increased sampling as well as elimination of noise in the single molecule data should improve the Gaussian fitting algorithm and further improve agreement between observed and predicted midpoints.

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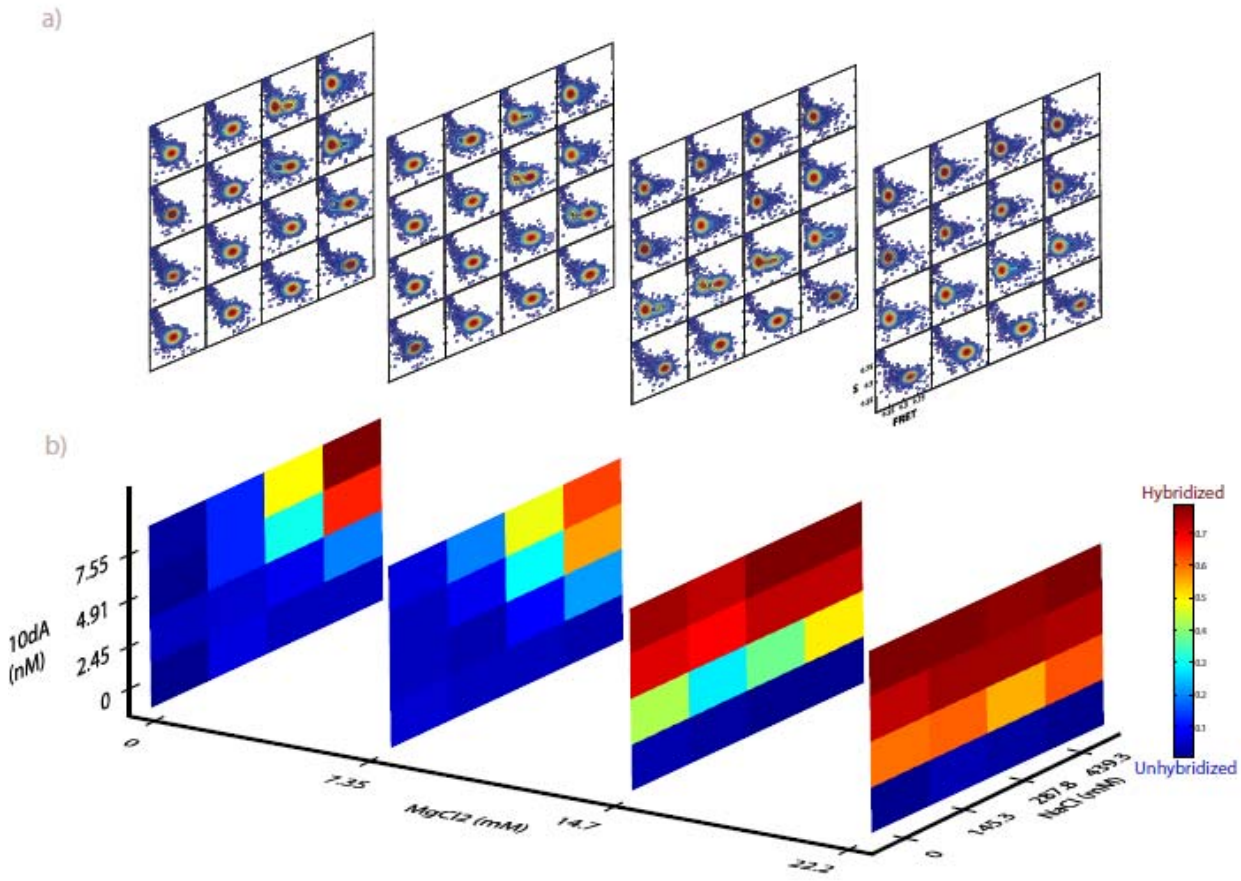

SUPPLEMENTARY FIGURE 7.

SUPPLEMENTARY FIGURE 7. A three-dimensional (4 x 4 x 4) exploration of physiochemical space (with complementary strand, monovalent and divalent salts) demonstrates the role of environment in hybridization and conformation of ssDNA. Poly(dT) was combinatorially titrated with 10 dA ssDNA, $MgCl_2$, and NaCl as indicated by the axes. The data represents a three-dimensional landscape of the hybridization efficiency of doubly labeled 20 dT ssDNA. Along with comparing the effects of the different reagents, the additive effects of reagents can be visualized with this method. (a) Raw ALEX data showing FRET and S values for every condition. (b) Processed heatmaps, color represents hybridization ratio as in Figure 1 in the main text.

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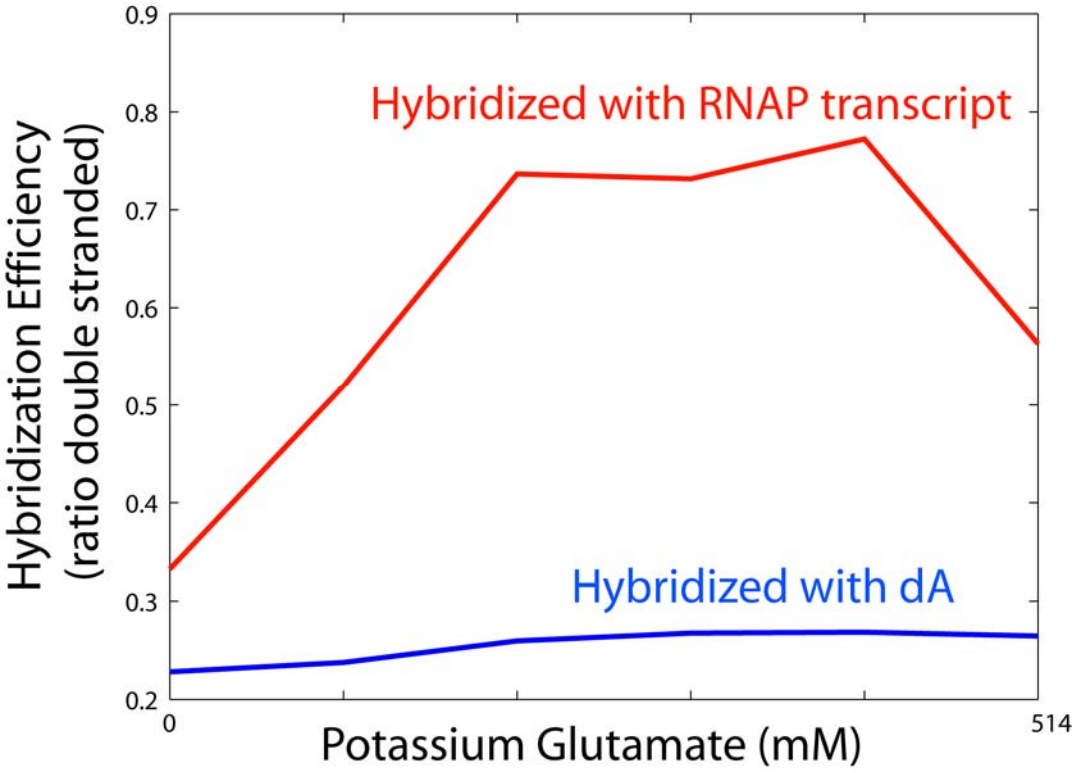

SUPPLEMENTARY FIGURE 8.

SUPPLEMENTARY FIGURE 8. The limited effect of glutamate on hybridization efficiency alone was probed with a 20 - bp length poly-A ssDNA (blue). This is compared with the pronounced effect of glutamate on RNAP transcription (red, data derived from column five of Figure 2**a**). Poly(dA) was measured at a concentration of 44 pM and the RNAP protein concentration was 569 pM; as with all other measurements, poly(dT) was measured at 250 pM.

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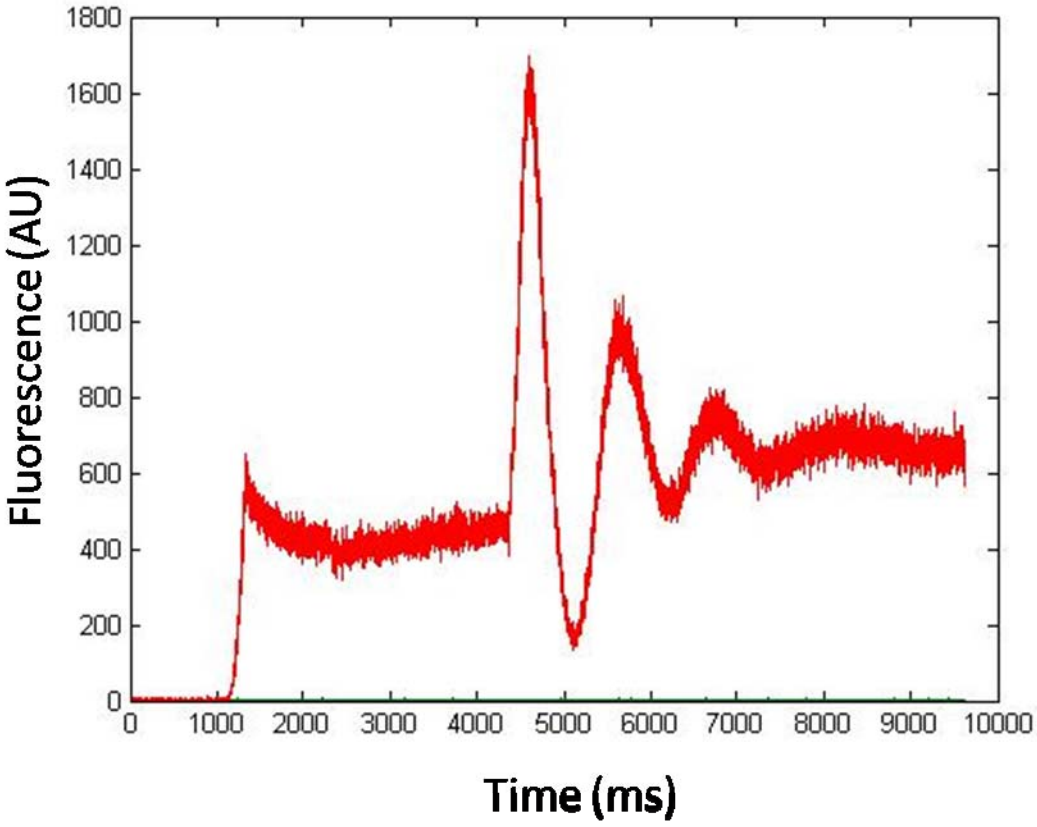

SUPPLEMENTARY FIGURE 9.

SUPPLEMENTARY FIGURE 9. The transient mixing response (reported as fluorescence intensity as function of time) following an initial injection (at ~1200 ms) of dye into the mixing ring of the formulator and subsequent iterations of mixing (in the window 4000 - 8000 ms). Steady state of mixing is achieved at ~10,000 ms (10 s).

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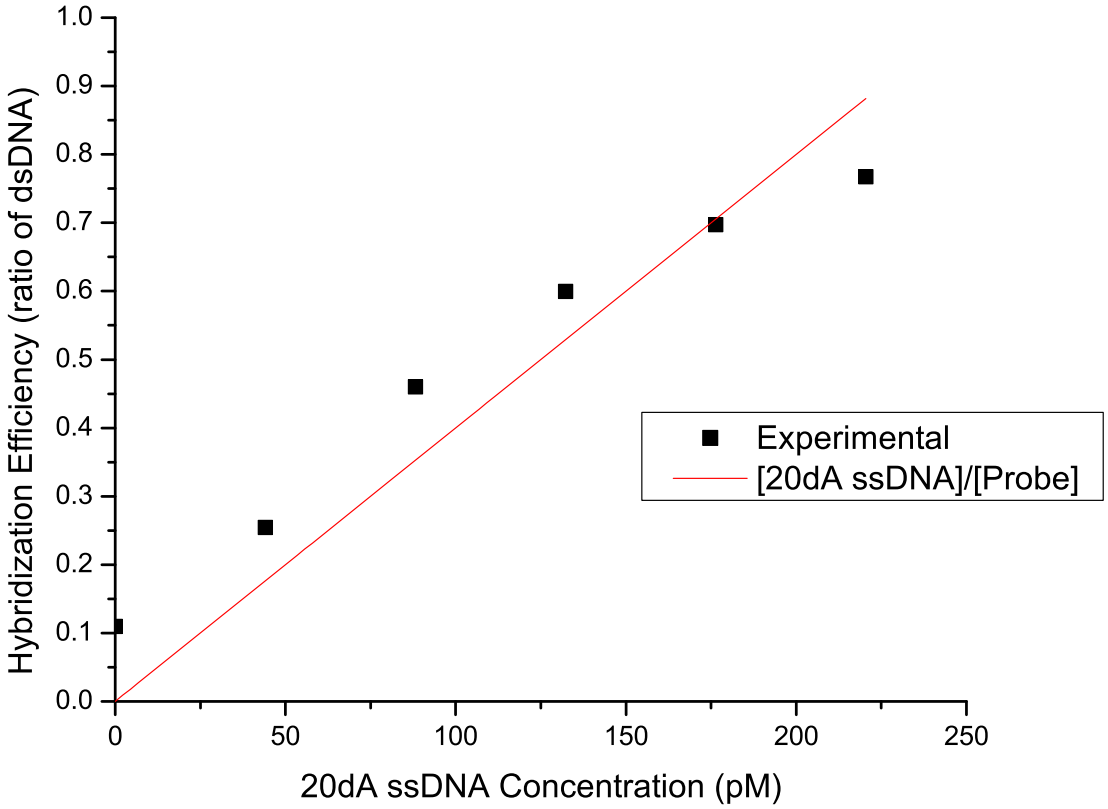

SUPPLEMENTARY FIGURE 10.

SUPPLEMENTARY FIGURE 10. The experimentally obtained dsDNA ratios, *R,* at a range of 20 dA ssDNA concentrations (black dots) are plotted with the with the ideal ratio, *Ri*, assuming complete hybridization (red line). *Ri* is determined by dividing experimental 20 dA ssDNA concentrations by the total 20 dT present (250 pM).

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SUPPLEMENTARY NOTE

The software suite included in the supplement of this paper contains software used in this study to acquire data and control microfluidics and optical components. In the root directory, “SM formulator controller.vi” is a LabView file used to control microfluidics alone. This file is associated with its own “README (1)” file and “Sample Protocol” to explain its use. The AutoCAD file of the formulator is included in this directory as “SM formulator.dwg.”

Also included in the directory “Labview Drivers” is a basic control routine implemented in Labview to control a microfluidic device using a home-made USB controller; its use is commented upon in a “README” file also in that directory.

Finally, in the directory, “ALEX Controller” is the software used to control and acquire single molecule data (controls laser pulses and collects single photon streams from avalanche photodiodes). It is a large suite of LabView files that also is explained with a “README” file also in that directory.

Software is also available at http://sourceforge.net/projects/uformulator/files/ where future updates may be deposited.

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Supplementary Table 1. Table of Primers Used In This Study

Oligo#1 (20 dT Probe)	ALEXA647-TTTTTTTTTTTTTTTTTTT-TAMRA'
Oligo#2 (Poly 10 dA)	5'-AAAAAAAAAA-3'
Oligo#3 (Poly 20 dA)	5'-AAAAAAAAAAAAAAAAAAAA-3'
DNA Template for Transcription (non-Template Strand)	5'- AGGCTTGACACTTTATGCTTCGGCTCGTATAATGTGTG GAATTGTGAGAGCGGAAAAAAAAAAAAAAAAAAAA-3'

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