SUPPLEMENTARY INFORMATION for

Speeding up the self-assembly of DNA nanodevice by a variety of polar solvents

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1. Methods

1.1 Materials. The DNA oligonucleotides used in System I and System II were purchased from Integrated DNA technologies (IDT). The DNA oligonucleotides used in System III were purchased from Biosearch Technologies, Inc. (Novato, CA, USA). DNA modified with dye and quencher were purified by HPLC, and others were purified by PAGE process. The DNA sequences are listed in **Supplementary Table S1**(*5*). Mfold was used to calculate the standard free energies of the DNA strands and complexes. Methanol, ethanol, glycol, glycerin and glucose and all other chemicals were of reagent grade or better and used as received. If not stated otherwise, the reaction buffer contains: 10 mM phosphate, 150 mM NaCl, 1 mM EDTA, and pH 7.2.

1.2 Fluorescence measurements. Fluorescence (Varian Cary Eclipse Fluorimeter, Varian, Inc.) was measured with a Peltier block, using quartz fluorescence cuvettes (4×10 mm; Sub-micro, 50 µl), and with the following settings: λ_{ex} = 556 nm, λ_{em} = 581 nm, 5 nm slit, PMT detector voltage = 760V. For the preparation of left or right arm labeled MP complex, stoichiometric amounts of 5'-TAMRA labeled P and 3'-Iowa Black labeled M strands were added to the reaction buffer to give a final concentration of 500 nM for each strand. The resulting solutions were heated to 90^{o} C for 5 min, and subsequently cooled to room temperature. Fluorescence measurements as a function of time were carried out by using the Varian Cary Eclipse Fluorimeter equipped with a temperature controller. The excitation wavelength was fixed at 556 nm, and the emission signal passing through a 581 nm filter was recorded by a fluorescence detector. In a typical test, 100 µL MP solution was added in a cuvette, and 1 µL of corresponding concentration of strand M' (if not stated otherwise, the final concentration is 1.75 µM) was added and mixed quickly within 20 s to initiate the reaction.

1.3 Surface Electro-chemistry Experiments. SM and SP were prepared using a well-established procedure described by Xiao et al.[23] In brief, prior to sensor fabrication, gold disk electrodes (2 mm diameter, CH Instruments, Austin, TX) were cleaned both mechanically (by polishing with diamond and alumina oxide slurries organic polar solvent assisted, successively) and electrochemically (through successive scans in sulfuric acid solutions) as previously described (*40*). The linear probe DNA was reduced for 1 hour at room temperature in the dark in 10 mM tris(2-carboxyethyl) phosphine hydrochloride (Molecular Probes, Carlsbad, CA) and then diluted to a final concentration of 1.0 µM in HEPES/NaClO4 buffer (10 mM HEPES and 0.5 M $NaClO_4$, pH 7.0, as was used in all the experiments surface electro-chemistry experiments unless otherwise noted. The gold electrodes were incubated in this solution for 1 hour at room temperature in the dark, rinsed with distilled, deionized (DI) water, and then incubated in 3 mM 6-mercapto-1-hexanol in DI water for 30 min. Following this, the electrodes were rinsed in DI water and stored in HEPES/$NaClO_4$ buffer for future use. Fabricated SM/SP were interrogated

using square wave voltammetry (SWV) with a 50 mV amplitude signal at a frequency of 60 Hz, in the absence and presence of SM' (2 μM). Signal gain was computed by the relative change in SWV peak currents with respect to background current.

Table S1. DNA sequences used in this work

Experiment in solution:

5'-3'

M with quencher:

CTGGAA TCGTCGTTTACGGTC CACACAGTAGATCAGAATTGGCACGTTCGCTCGCTAGGTT GAAGTCACCCTCATT –Q

P with dye:

F-AATGAGGGTGACTTC GACCGTAAACGACGA

M':

AATGAGGGTGACTTC AACCTAGCGAGCGAACGTGCCAATTCTGATCTACTGTGTG GACCGTAAACGACGA

P':

TCGTCGTTTACGGTC GAAGTCACCCTCATT

M' with dye:

F-AATGAGGGTGACTTC AACCTAGCGAGCGAACGTGCCAATTCTGATCTACTGTGTG GACCGTAAACGACGA

The sequences for strands listed above are the same as in previous work.[1]

Experiment on electrode:

5'-3'

SP:

TTTTTTTTTTTTTTTGCATCCACTCATTCAATACC-MB

SM':

CACTCATTCAATACCCTACGTC

SM:

SH-GACGTAGGGTATTGAATGAGTGGATGC

F: TAMRA
Q: Iowa Black
MB: Methylene Blue

Figure S1. The performance of dye (TAMRA) in alcohols

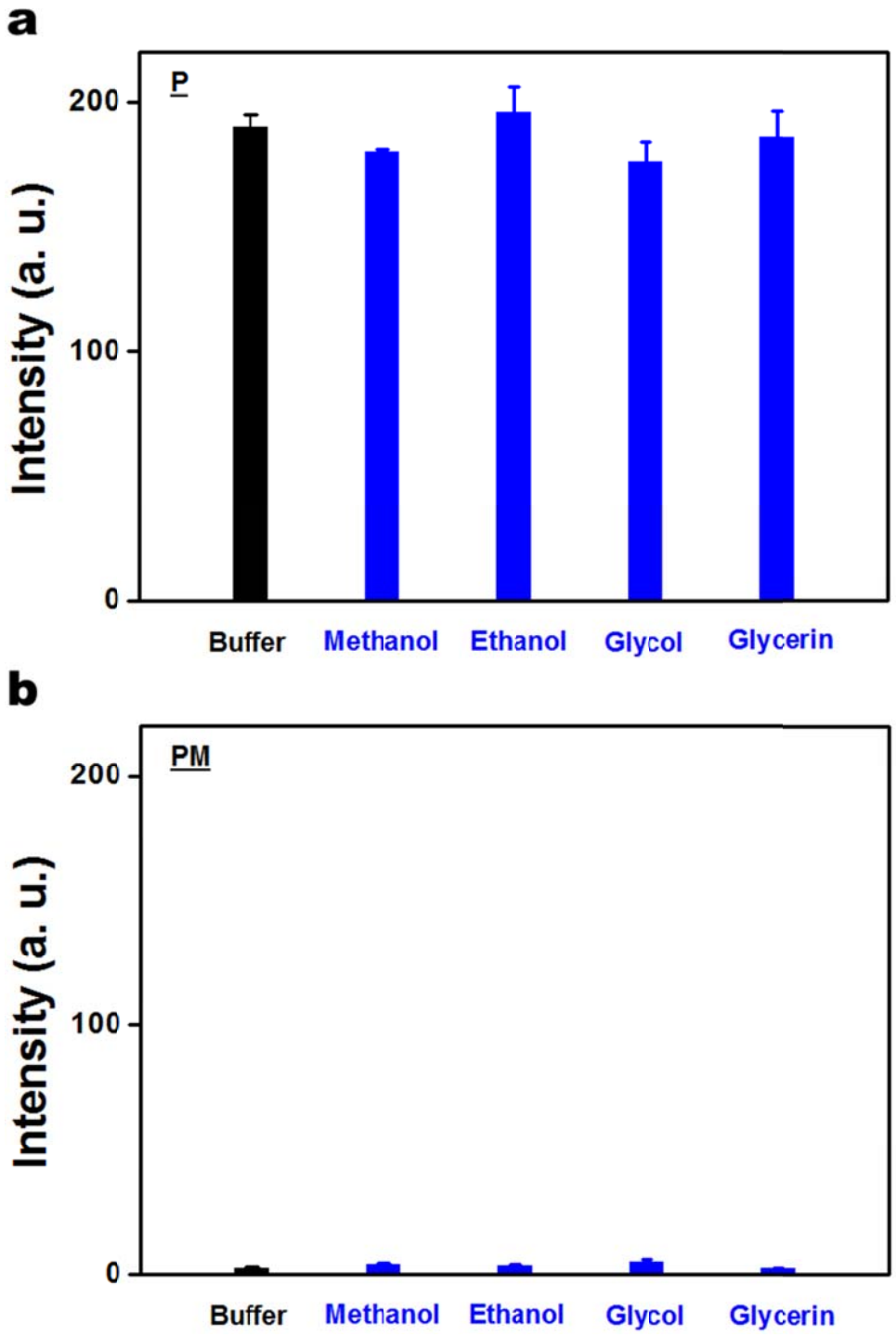


Figure S1. a, The fluorescence intensity of strand P (500 nM) modified with dye in pure aqueous buffer and the mixed solution containing different polar solvents (20%). The nearly equal values of the fluorescence indicate that the organic solvents have minimal effect on the fluorescence intensity. **b,** The M (1750 nM) is hybridized with P (500 nM) so that the fluorophore and quencher have been brought in close proximity. The fluorescence intensity is that of the fluorophore which is present in the same concentration and in the same buffer compositions as in (a).

Figure S2 MP and MM' melting curves in buffer

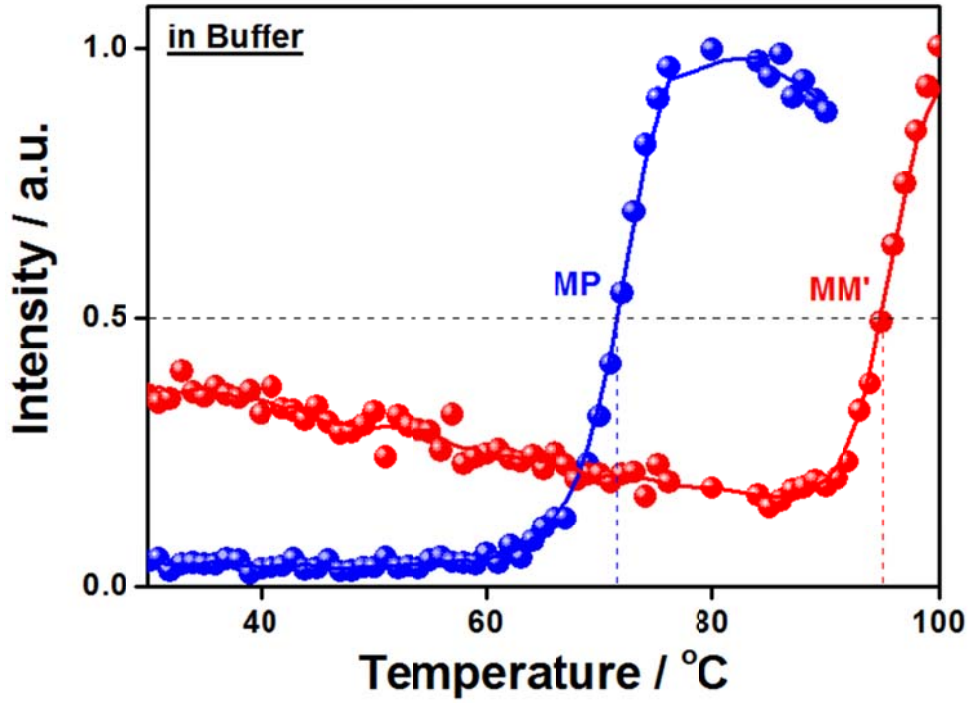


Figure S2. Melting curves of MP and MM' in buffer. The buffer is 10 mM phosphate, 150 mM NaCl, 1 mM EDTA and pH 7.2.

Figure S3 MP and MM' melting curves in 10% ethanol

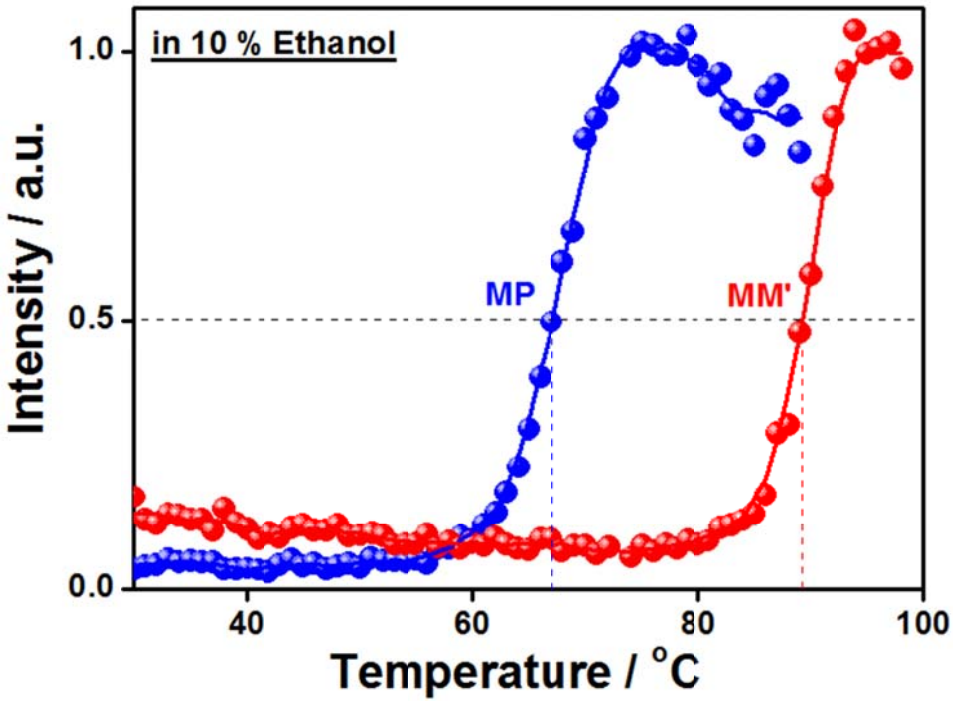


Figure S3. Melting curves of MP and MM' in 10% ethanol. The buffer is 10 mM phosphate, 150 mM NaCl, 1 mM EDTA, and pH 7.2.

Figure S4 MP and MM' melting curves in 20% ethanol

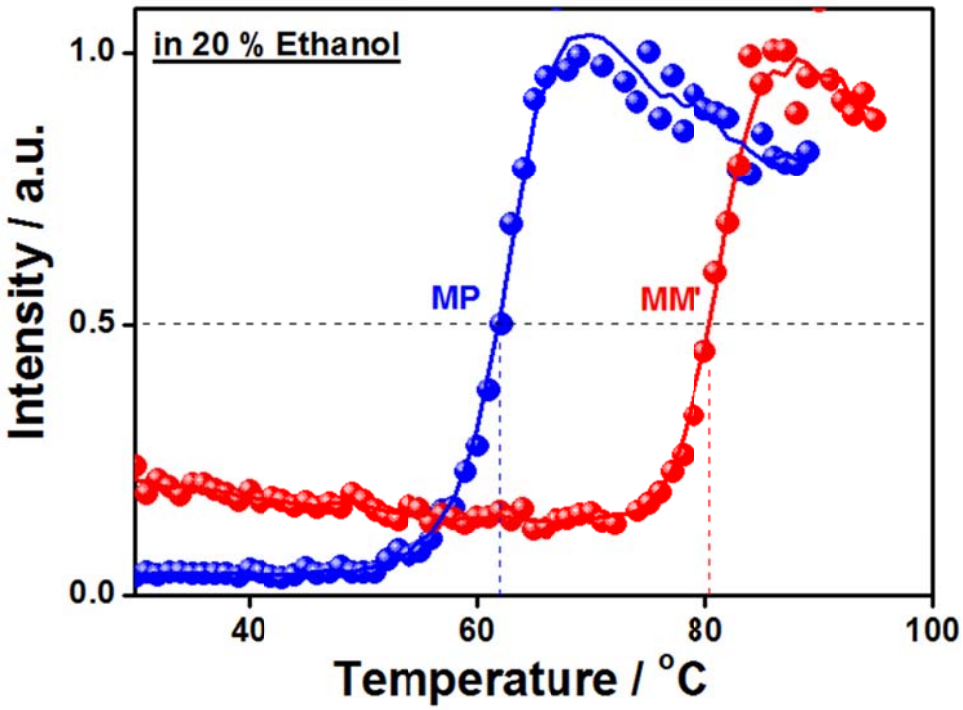


Figure S4. Melting curves of MP and MM' in 20% Ethanol. The buffer is 10 mM phosphate, 150 mM NaCl, 1 mM EDTA, and pH 7.2.

Figure S5 MP and MM' melting curves in 30% ethanol

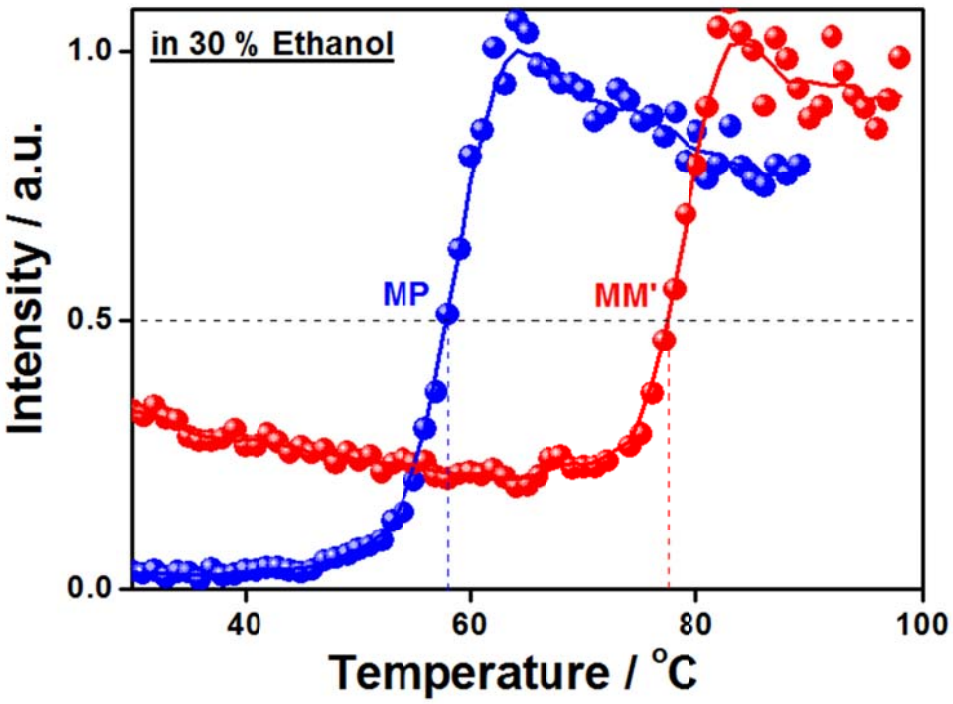


Figure S5. Melting curves of MP and MM' in 30% ethanol. The buffer is 10 mM phosphate, 150 mM NaCl, 1 mM EDTA, and pH 7.2.

Figure S6. MP and MM' (M: 100 nM) melting curves

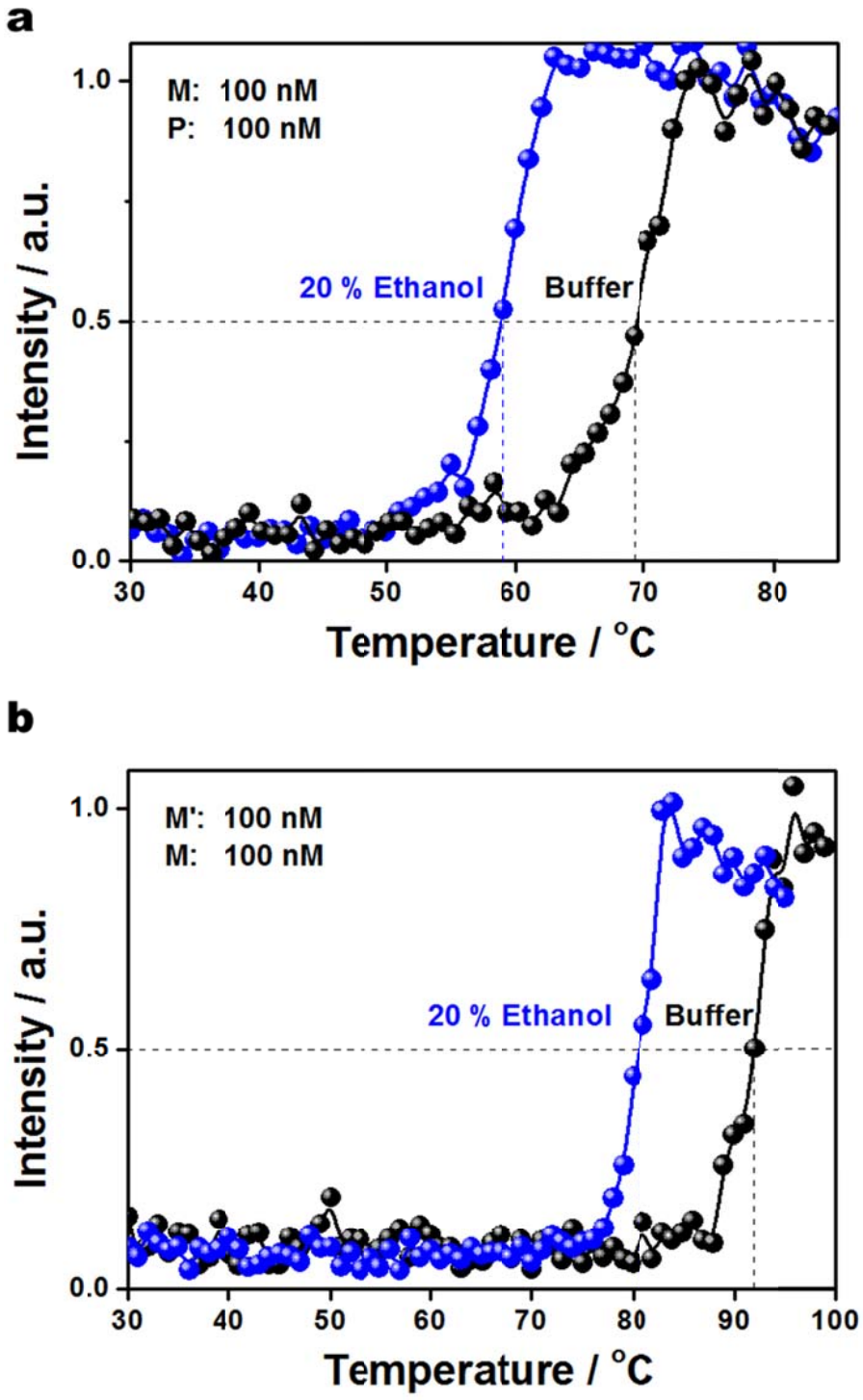


Figure S6. Melting curves of MP (100 nM + 100 nM) and MM' (100 nM + 100 nM) in buffer and 20% ethanol. The buffer is 10 mM phosphate, 150 mM NaCl, 1 mM EDTA, and pH 7.2.

Figure S7. MP and MM' (M: 500 nM) melting curves

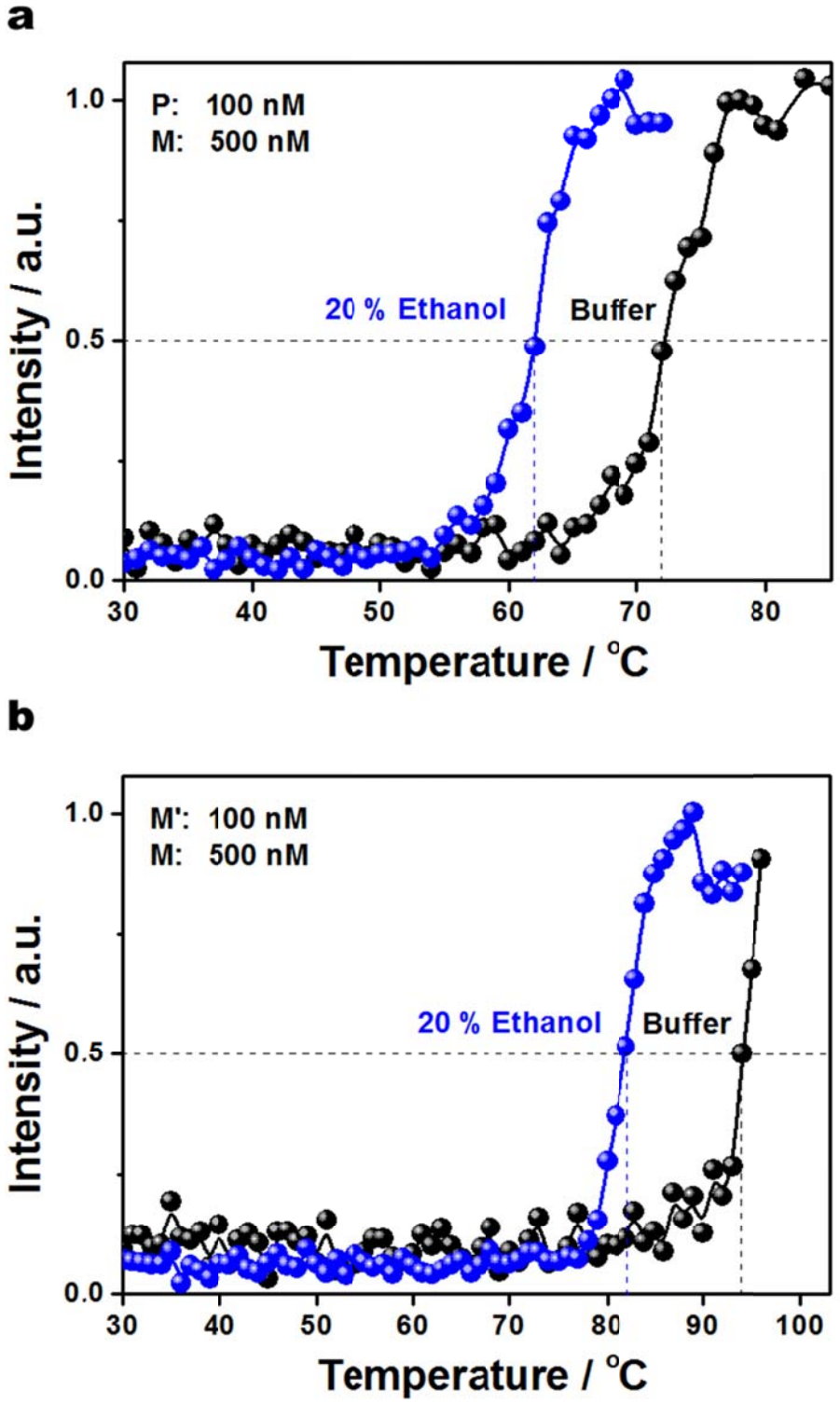


Figure S7. Melting curves of MP (500 nM + 100 nM) and MM' (500 nM + 100 nM) in buffer and 20% ethanol. The buffer is 10 mM phosphate, 150 mM NaCl, 1 mM EDTA, and pH 7.2.

Figure S8. MP and MM' (M: 1000 nM) melting curves

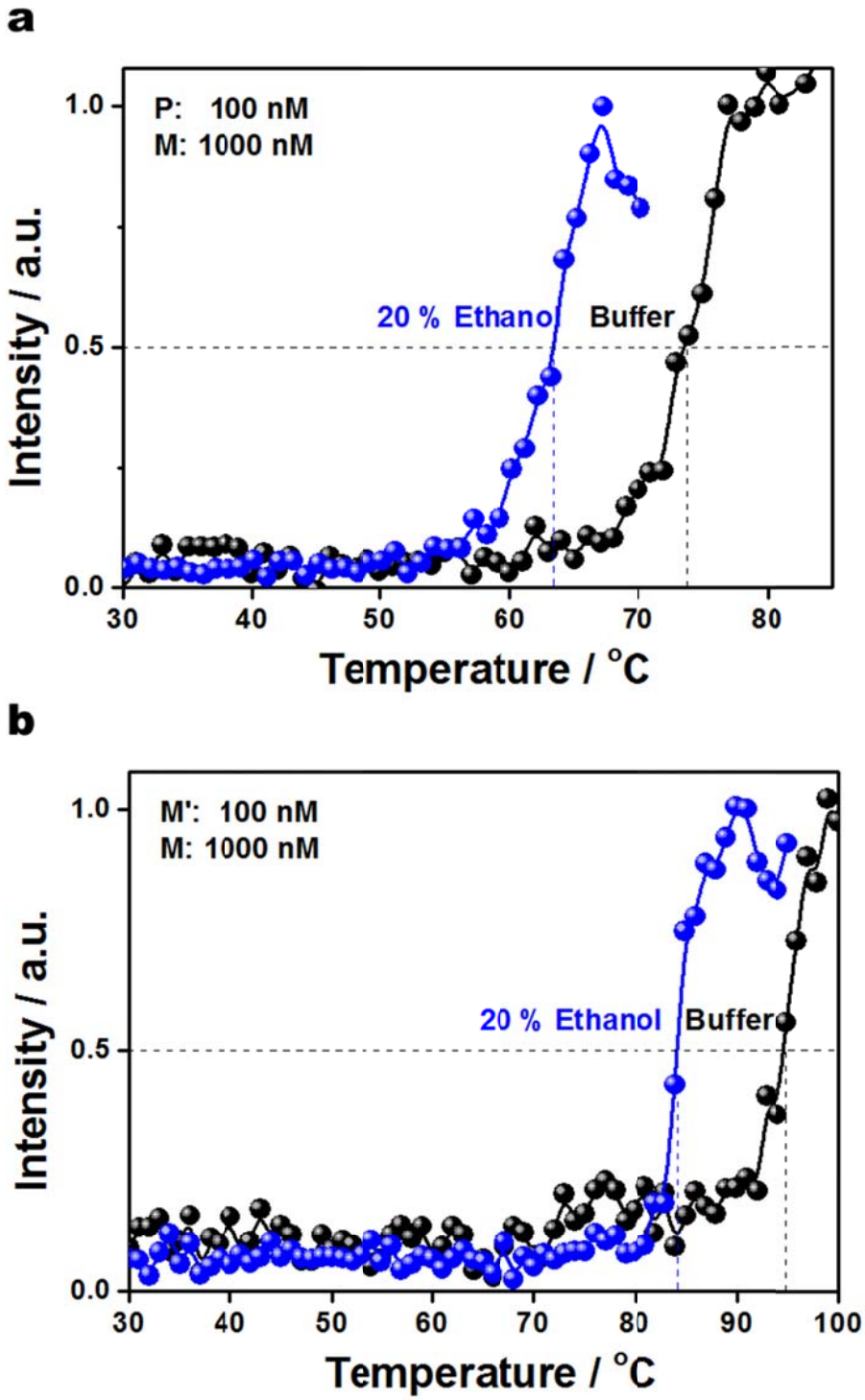


Figure S8. Melting curves of MP (1000 nM + 100 nM) and MM' (1000 nM + 100 nM) in buffer and 20% ethanol. The buffer is 10 mM phosphate, 150 mM NaCl, 1 mM EDTA, and pH 7.2.

Figure S9. MP and MM' (M: 1750 nM) melting curves

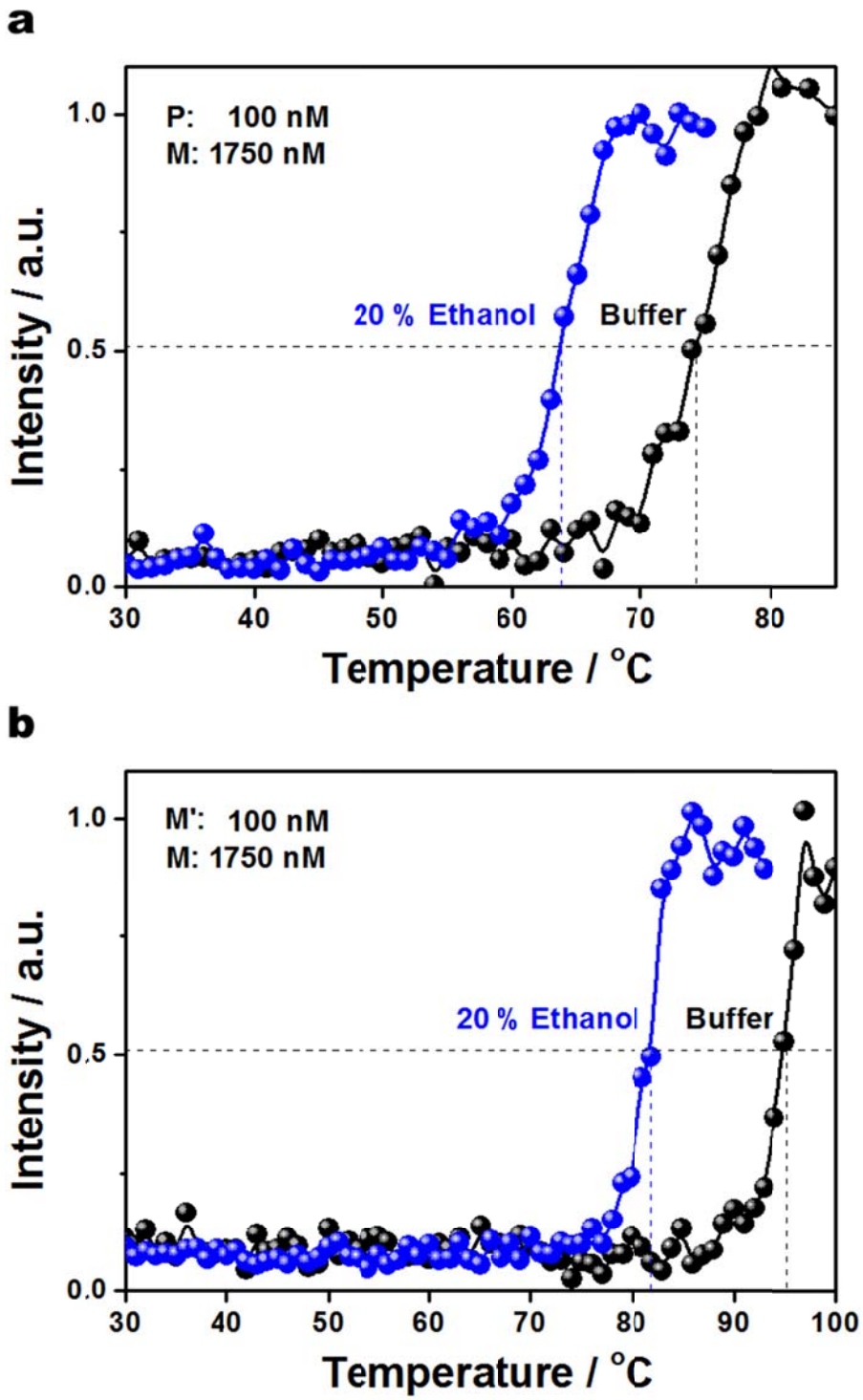


Figure S9. Melting curves of MP (1750 nM + 100 nM) and MM' (1750 nM + 100 nM) in buffer and 20% ethanol. The buffer is 10 mM phosphate, 150 mM NaCl, 1 mM EDTA, and pH 7.2.

Figure S10. MP and MM' (M: 3500 nM) melting curves

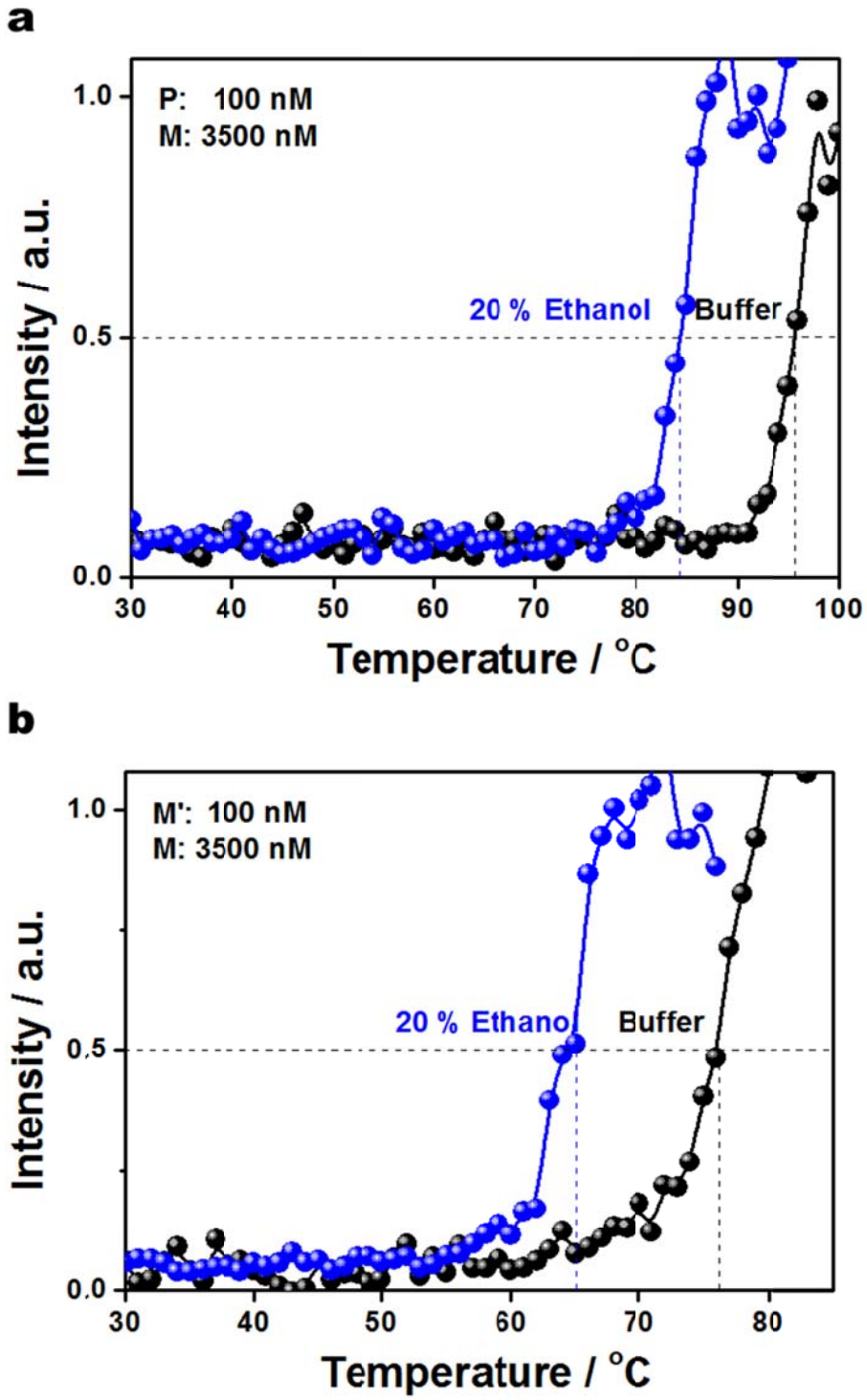


Figure S10. Melting curves of MP (3500 nM + 100 nM) and MM' (3500 nM + 100 nM) in buffer and 20% ethanol. All the samples contain 10 mM phosphate, 150 mM NaCl, 1 mM EDTA, and pH 7.2.

Text S1. Calculation of thermodynamic parameters.

Consider the general equilibrium shown below for the association of DNA sequences in our work to form a duplex structure.

$$M + P \leftrightarrow MP \quad (1)$$

$$M + M' \leftrightarrow MM' \quad (2)$$

The general expression for the corresponding equilibrium constant K is ((1) as an example)

$$K = \frac{[MP]}{[M][P]} \quad (3)$$

We now have an expression for the equilibrium constant in terms of experimentally accessible parameters (concentrations). If we define the melting temperature Tm, as the temperature at which [MP] is 0.5[P] (since [M] ≥ [P]), then the general expression for the equilibrium constant shown in Eq (3) reduces to

$$K_{T_m} = \frac{0.5[P]_0}{([M]_0 - 0.5[P]_0)0.5[P]_0} = \frac{1}{([M]_0 - 0.5[P]_0)} \quad (4)$$

Since, for any process at equilibrium, $\Delta G^0 = -RT \ln K_{eq}$ and $\Delta G^0 = \Delta H^0 - T\Delta S^0$, we can derive an expression for K_{eq} in terms of ΔH^0 and ΔS^0 by equating these two expressions for ΔG^0 to yield

$$-RT \ln K = \Delta H^0 - T\Delta S^0 \quad (5)$$

Equation (4) provides us with an expression for K at T_m in terms of $[M]_0$ and $[P]_0$ (the original concentrations) when double strands associate. Plugging this expression into the equality given above yields

$$+ RT_m \ln([M]_0 - 0.5[P]_0) = \Delta H^0 - T_m \Delta S^0 \quad (6)$$

Dividing by T_m and rearranging the terms yields

$$R \ln([M]_0 - 0.5[P]_0) = \Delta H^0 \frac{1}{T_m} - \Delta S^0 \quad (7)$$

(Y) (a) (X) (b)

As emphasized by the symbols in parentheses, this equation corresponds to a straight line when $R \ln([M]_0 - 0.5[P]_0)$ is plotted against the reciprocal of the melting temperature ($1 / T_m$). The slope (a) of such a plot is equal to ΔH^0 and the intercept (b) is equal to $-\Delta S^0$. Figure S13 shows a typical $R \ln([M]_0 - 0.5[P]_0)$ vs ($1 / T_m$) plot in which these features are emphasized.

From Figure S13, we could know the (ΔH^0) and (ΔS^0), and we could get the following Table S3.

Since the single-stranded state for M' and P is random coil state, their ΔG is considered to be 0. We therefore have Figure S14, which shows the initiate state and final state of DNA Strand Displacement Reaction.

Table S2. ΔH^0, ΔS^0, and ΔG (at 25° C)

Category	ΔH^0 / kcal mol^{-1}	ΔS^0 / cal mol^{-1} k^{-1}	ΔG / kcal mol^{-1}
MM' in buffer	279.8	733.3	-61.4
MM' in ethanol	206.1	550.5	-42.1
MP in buffer	151.0	410.8	-28.8
MP in ethanol	134.8	372	-23.9

Table S2. To calculate ΔG difference of M/P and M/M' in solvent (e.g. 20% ethanol) from those in aqueous buffer, we measured T_m in solvent and buffer system with various M/P and M' concentrations. Then we plotted R ln ($[M]_0$ - 0.5$[P]_0$) against the reciprocal of the melting temperature (1 / T_m) to get (ΔH_0) and (ΔS_0) to calculate ΔG.

Table S3. Displacement ratio of System I in 60s, 300s, and 600s in alcohols

Category	Buffer	30% Methanol	30% Ethanol	30% Glycol
DR in 60s	**9%**	37%	57%	**62%**
DR in 300s	**22%**	48%	72%	76%
DR in 600s	**31%**	57%	73%	77%

Table S3. DSDR in System I in buffer and different polar solvents. DSDR in buffer solution completes only 9% in 60 seconds, 22% in 300 seconds, and 31% in 600 seconds. DSDR in 30% methanol, ethanol, and glycol solutions completes 37%, 57%, and 62% replacement reactions, respectively, in first 60 seconds, which indicates the polar solvent/buffer (glucose/buffer) co-solvents highly increased the displacement ratio in the very beginning of DSDR.

Table S4. Displacement ratio of System II in 60s, 300s, and 600s in alcohols

Category	Buffer	20% Ethanol
DR in 60s	**10%**	30%
DR in 300s	**20%**	64%
DR in 600s	**42%**	**70%**

Table S4. DSDR in System II in buffer and Ethanol solvents. DSDR in buffer solution completes only 10% in 60 seconds, 20% in 300 seconds, and 42% in 600 seconds. DSDR in 20% Ethanol solution completes 70% replacement reactions in the first 600 seconds which indicates the alcohol/buffer co-solvents highly increased the displacement ratio of DSDR on the surface.

Figure S11. DSDR in buffer at various temperatures

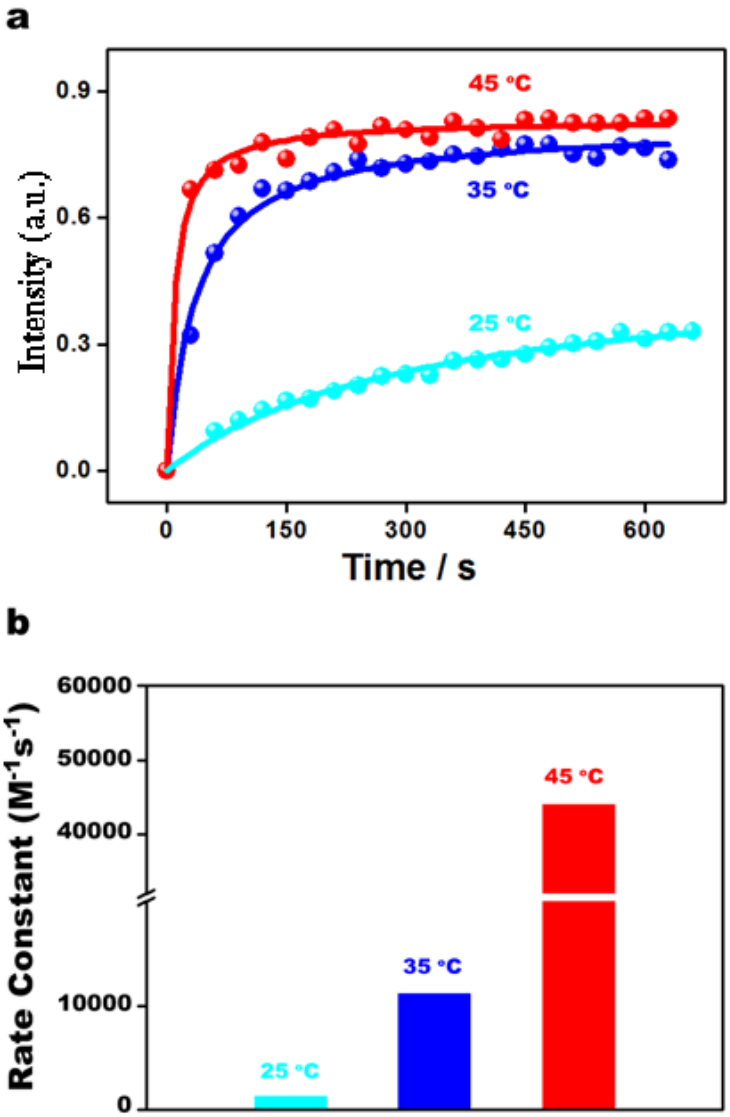


Figure S11. a, Fluorescence spectroscopy test of DSDR in buffer solution at different temperatures. MP in solution (100 µL, 500 nM) is placed in a cuvette, and strand M' (1 µL) is added and mixed quickly to initiate the DSDR. The final concentration of strand M' is 1.75 µM (1: 3.5) at 25° C, 35° C and 45° C, respectively. **b,** is the K constant for DSDR in buffer between M' and MP at 25° C, 35° C, and 45° C, respectively.

Figure S12. DSDR in 20% ethanol at various temperatures

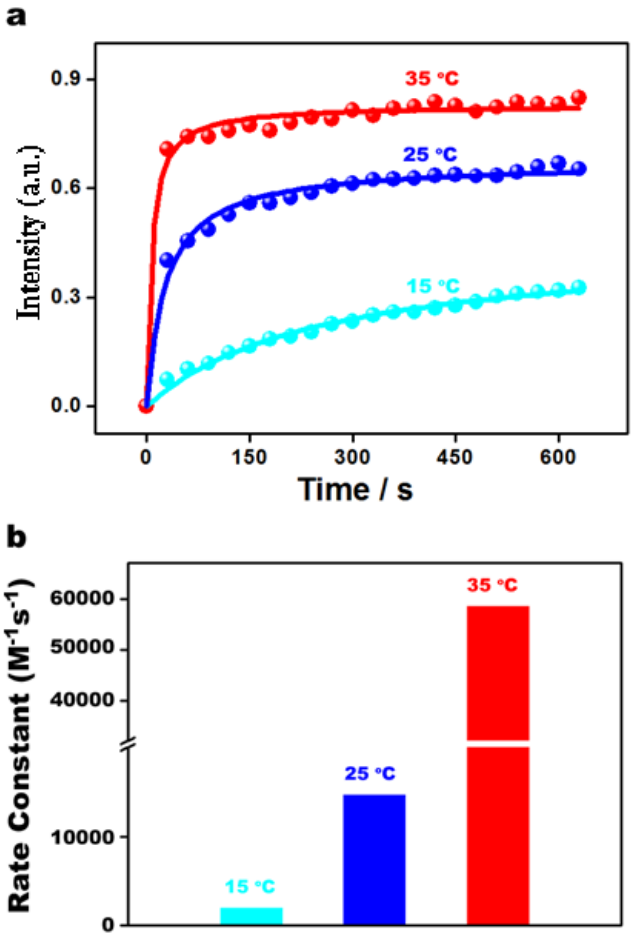
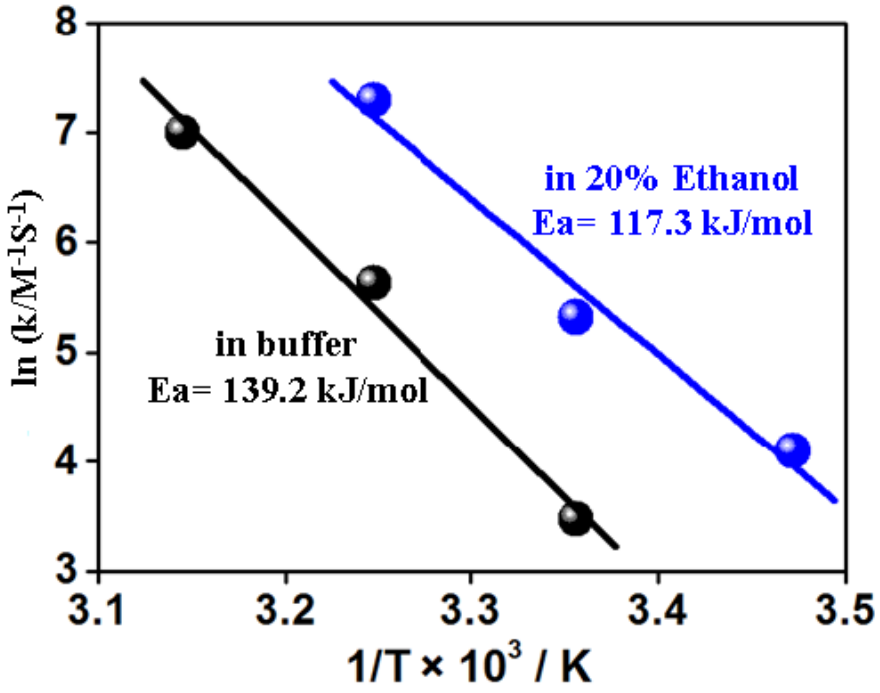


Figure S12. a, Fluorescence spectroscopy test of DSDR in 20% Ethanol solution at different temperatures. MP in solution (100 μL, 500 nM) is placed in a cuvette, and strand M' (1 μL) is added and mixed quickly to initiate the DSDR. The final concentration of strand M' is 1.75 μM (1 : 3.5) at 15° C, 25° C, and 35° C, respectively. **b,** is the rate constant for DSDR in 20% ethanol solution between M' and MP at 15° C, 25° C, and 35° C, respectively.

Figure S13. Arrhenius Plots



$$\Delta Ea = E_{a\ buffer} - E_{a\ 20\%Ethanol} = 21.9\ kJ/mol$$

Figure S13. Arrhenius plots for strand exchange reaction in 20% ethanol or buffer. Values of activation energies (Ea) calculated from the Arrhenius plots are shown above.

Table S5. $t_{1/2}$ in System II

	20%
BUFFER	**1000 s**
Ethanol	**85 s**

Table S5. $t_{1/2}$ of DSDR on surface in buffer is 1000 seconds. $t_{1/2}$ of 20% ethanol is only 85 seconds in System II. These experiments demonstrate that our organic polar solvent assisting strategy not only works in solution but also on surface.