Enhanced DNA Toehold Exchange Reaction on Chip Surface to Discriminate Single-Base Changes

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Supporting Information

Supporting information text

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Materials. Ultrapure water with an electrical conductivity of 18.2 MΩ·cm (Millipore Corporation, MA, USA) was used in all experiments. Chemical reagents were of analytical grade and used without further purification. DNA oligonucleotides were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China) and purified by high-performance liquid chromatography (HPLC) or ultraPAGE. The DNA sequences are shown in Table S1. TE/Mg^{2+} buffer (10 mM Tris–HCl, 12.5 mM $MgCl_2$; pH 8) and phosphate-buffered saline (10 mM PBS, 0.3 M NaCl; pH 7.4) were used in all experiments. The probe *PC* was prepared by mixing *P* and *C* in TE/Mg^{2+} buffer. The solution was annealed in a polymerase chain reaction (PCR) instrument (TC-5000, Techne, Staffordshire, UK). The temperature of the samples was reduced from 95 °C to room temperature for 90 min at a constant rate of 0.02 °C/s.

Dual polarization interferometry (DPI) experiments. The toehold exchange on the chip surface was evaluated in real time by DPI (AnaLight Bio200, Farfield Group Ltd., Stockholm, Sweden) as previously described.[1, 2] A He–Ne laser that emits

light at 632.8 nm was used to measure optical phase changes. Light polarization was switched between transverse-electric (T_E) and transverse-magnetic (T_M) using a polarizer. The fluidic system and instrument consisted of an HPLC injector valve and an external pump (PHD2000, Harvard Apparatus, MA, USA) that provides a controlled continuous fluid flow over the two channels on the chip surface. All DPI experiments were performed at 20 ± 0.002 °C. An amine-modified sensor chip (FB 100 Amine, Farfield Group, Ltd., Stockholm, Sweden) was mounted on the instrument. Before each experiment, the sensor chip was calibrated by using an 80% (w/w) ethanol/water solution and ultrapure water. The refractive indices (RIs) of these media were used to calibrate the RIs of PBS and TE/Mg^{2+} buffer.

After calibration, the flow rate was changed to 25 μL min^{-1}. Glutaraldehyde (4 mg mL^{-1} in PBS) and NeutrAvidin protein (31000, Thermo Fisher Scientific, MA, USA; 0.5 mg mL^{-1} in PBS) solutions were injected into each channel for 6 min. After injecting NeutrAvidin protein, the buffer was changed from PBS to TE/Mg^{2+}. Then, the TE/Mg^{2+} buffer was allowed to flow on both channels for 20 min at 50 μL min^{-1} to ensure baseline stabilization. *PC* was prepared at a concentration of 1 μM in TE/Mg^{2+} buffer and then allowed to flow through the surface at 10 μL min^{-1} for 15 min before it returned to the TE/Mg^{2+} buffer. The immobilization process is illustrated in Scheme 1 (Steps 1 to 3).

The flow rate was maintained at 10 μL min^{-1} after *PC* was immobilized and stabilized on the chip surface. Different spurious target (*S*) solutions and the correct target (*X*) solution at 1 μM in TE/Mg^{2+} buffer were injected into both channels to

investigate toehold exchange. The phase changes of T_E and T_M were recorded in real time. The absolute layer thickness and RI were directly resolved from the T_M and T_E phase values by using an analysis software. Layer mass was quantified according to the following De Feijter equations:

$$\rho_L = \frac{n_L - n_{buffer}}{dn/dc} \quad (1)$$

$$m_L = \rho_L \cdot \tau_L \quad (2)$$

where m_L is the layer mass per unit area (ng mm^{-2}), τ_L is the layer thickness (nm), n_L and n_{buffer} are the RIs of the adsorbed layer and the bulk solution, respectively, and *dn/dc* is the RI increment of the DNA (cm^3 g^{-1}). A *dn/dc* value of 0.183 was used according to Tumoloet et al.[4] The toehold exchange efficiency on the chip surface was calculated as follows:

$$\emptyset = \frac{\Delta m_L \ \ M(PC)}{m_L(PC)\ M(C)} \times 100 \quad (3)$$

where $\emptyset$ is the toehold exchange efficiency on the chip surface, Δm_L is the decrease in mass upon the addition of different targets, $m_L(PC)$ is the mass of the immobilized probe *PC*, *M* (PC) is the molecular weight of the probe *PC*, and *M* (C) is the molecular weight of the complement *C*.

Fluorescence test of toehold exchange in solution. For the fluorescence test, a 1.1:1 ratio of *P-quencher* to *C-ROX* in TE/Mg^{2+} buffer was used to prepare the *PC*. The solution was annealed in the PCR instrument. *X* or *S* was added to the annealed *PC* solution. Toehold exchanges proceeded at 20 °C for 1 h. Then, an F-7000 spectrofluorometer (Hitachi Ltd., Tokyo, Japan) was used for all steady-state

operations at an excitation wavelength of 588 nm. The toehold exchange efficiency was calculated as follows:

$$\emptyset = \frac{F_{X\ or\ S}}{F_{C-ROX}} \times 100 \quad (4)$$

where $\emptyset$ is the toehold exchange efficiency in the solution, $F_{X\ or\ S}$ is the fluorescence intensity of the solution after toehold exchange upon the addition of *X* or *S*, and F_{C-ROX} is the fluorescence intensity of pure *C-ROX* with the same concentration as *C-ROX* in the reaction solution.

Native polyacrylamide gel electrophoresis (PAGE) analysis. The toehold exchange in the solution was observed using native PAGE. The gel was run in a 10% acrylamide (19/1 acrylamide/bisacrylamide) solution with 1×TAE buffer at a constant voltage of 100 V for 1 h. In a typical experiment, *PC* was prepared with a 1.1:1 ratio of *P-tail* to *C* in TE/Mg^{2+} buffer. Then, the solution was annealed in the PCR instrument. To distinguish DNA bands, a 15 T segment was added to *P* to increase the molecular weight of *PC*. *X* or *S* was then added to achieve the final concentrations of 2 μM for *X* or *S*, 1 μM for *PC*, and 0.1 μM for *P*. Toehold exchange proceeded at 20 °C for 1 h. The gel was run at room temperature and then stained with GelRed (Biotium Inc., California, USA) for 15 min to reveal DNA position.

Detection of single-base changes by fluorescence microscopy. An aldehyde-modified glass substrate was used in all experiments. In a typical experiment, the glass slide was cleaned with a 10% NaOH solution for 20 min at ~100 °C and then incubated in a hot piranha solution (H_2O_2/H_2SO_4, 3:7,v/v) for 30 min. Afterward, the glass slide was rinsed with ultrapure water and then blow-dried with N_2. The clean

glass slide was silanized with a 4% (v/v) (3-aminopropyl)-trimethoxysilane solution in isopropanol at room temperature for 2 h. The glass slide was again rinsed with isopropanol, blow–dried with N_2, and then baked at 120 °C for 30 min. Finally, the aminated glass slide was treated with a 5% glutaraldehyde aqueous solution for 4 h to obtain the aldehyde-modified glass slide.

A gasket with 12 square cells was stuck on the aldehyde-modified glass slide surface to obtain 12 reaction wells on the surface. Afterward, a NeutrAvidin protein solution (0.5 mg mL^{-1} in PBS) was added into the reaction wells and incubated for 3 h at 20 °C to react with the aldehyde group. After NeutrAvidin protein immobilization, *PC* (1 μM in TE/Mg^{2+} buffer) was added into the NeutrAvidin protein-modified reaction wells and then incubated for 1 h at 20 °C to immobilize *PC*. To detect single-base changes, toehold exchange was initiated by adding *X* or *S* (1 μM in TE/Mg^{2+} buffer) into the *PC*-modified reaction wells and then incubating for 1 h at 20 °C. After toehold exchange, the reaction wells were washed thrice with TE/Mg^{2+} buffer. A FAM-modified reporter solution (1 μM in TE/Mg^{2+} buffer) was added and then incubated for 1 h at 20 °C. Excess strands were removed with TE/Mg^{2+} buffer at room temperature. Fluorescent images were obtained using an inverted fluorescence microscope (IX71, Olympus Corporation, Tokyo, Japan).

Table S1. Detailed sequence of all oligonucleotide probes.

Name	Sequence
P	5′-**biotin**-TTTTTGCATCCACTCATTCAATACC-3′
Complement C	5′-ACGTAGGGTATTGAATGAGTGGATGC-3′

P-tail	5′-TTTTTTTTTTTTTTTGCATCCACTCATTCAATACC-3′
P-quencher	5′-TTTTT-**BHQ2**-GCATCCACTCATTCAATACC-3′
C-ROX	5′-ACGTAGGGTATTGAATGAGTGGATGC-**ROX**-3′
Correct target	5′-CACTCATTCAATACCCTACGT-3′
Correct target (6/6)	5'-ACTCATTCAATACCCTACGT-3′
m1T	5′-**T**ACTCATTCAATACCCTACGT-3′
m1T (6/6)	5′-**T**CTCATTCAATACCCTACGT-3′
m6T	5′-CACTC**T**TTCAATACCCTACGT-3′
m11T	5′-CACTCATTCA**T**TACCCTACGT-3′
m11G	5′-CACTCATTCA**G**TACCCTACGT-3′
m11C	5′-CACTCATTCA**C**TACCCTACGT-3′
i11A	5′-CACTCATTCA**A**ATACCCTACGT-3′
i11T	5′-CACTCATTCA**T**ATACCCTACGT-3′
i11G	5′-CACTCATTCA**G**ATACCCTACGT-3′
i11C	5′-CACTCATTCA**C**ATACCCTACGT-3′
d11	5′-CACTCATTCATACCCTACGT-3′
m16T	5′-CACTCATTCAATACC**T**TACGT-3′
m19T	5′-CACTCATTCAATACCCTA**T**GT-3′
m19A	5′-CACTCATTCAATACCCTA**A**GT-3′
m19G	5′-CACTCATTCAATACCCTA**G**GT-3′
i19A	5′-CACTCATTCAATACCCTA**A**CGT-3′
i19T	5′-CACTCATTCAATACCCTA**T**CGT-3′
i19G	5′-CACTCATTCAATACCCTA**G**CGT-3′
i19C	5′-CACTCATTCAATACCCTA**C**CGT-3′
d19	5′-CACTCATTCAATACCCTAGT-3′
FAM-reporter	5′-**FAM**-GGTATTGAATGAGTG-3′
New P	5′-**biotin**-TTTTTATGATTGAGGAAGTAGTTTG-3′
New Complement C	5′-ACTGTCCAAACTACTTCCTCAATCAT-3′
New correct target	5′-TGAGGAAGTAGTTTGGACAGT-3′

New-m6T	5′-TGAGGTAGTAGTTTGGACAGT-3′
New-m16T	5′-TGAGGAAGTAGTTTGTACAGT-3′

Table S2. Calculated toehold-exchange efficiencies on the chip surface and in the solution.

Targets		Correct	m1T	m6T	m16T	m11T	i11A	d11	m19T	i19A	d19
On chip surface	Ø	86.1	13.9	11.9	11.7	11.1	9.0	10.8	9.5	8.9	9.1
	ΔØ	/	72.2	74.2	74.4	75	77.1	75.3	76.6	77.2	77
In solution	Ø	24.9	9.1	4.8	4.8	4.2	5.8	4.4	5.6	5.7	6.6
	ΔØ	/	15.8	20.1	20.1	20.7	19.1	20.5	19.3	19.2	18.3

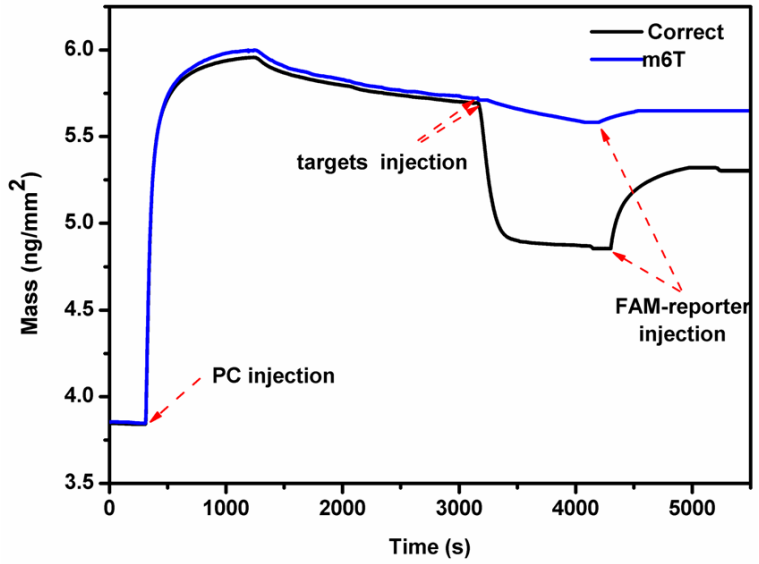

Figure S1. Real-time DPI measurements of surface-mass changes during the immobilization of dsDNA probe (*PC*) and the subsequent injections of different targets and FAM-modified reporter. The mass of the immobilized dsDNA probe $m_L(PC)$ is 1.8112 ng/mm^2. The mass decrease Δm_L is 0.7498 ng/mm^2 upon the addition of correct target ssDNA (Correct), and the toehold exchange efficiency is 86.1% (black curve). The mass decrease $\Delta m_L$is 0.1093 ng/mm^2 upon the addition of spurious target ssDNA (m6T), and the toehold exchange efficiency is 11.9% (blue curve). The ssDNA and FAM-modified reporter were added, and the surface mass

increased because it can directly bind with *P*.

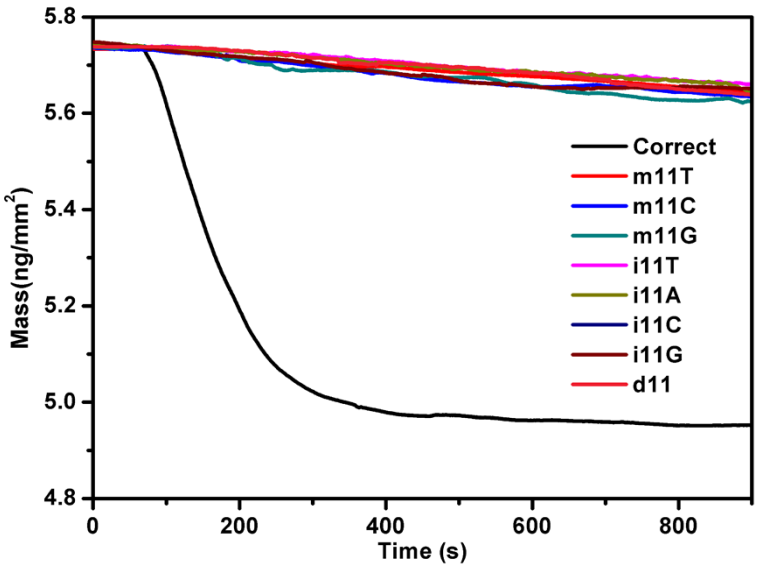

Figure S2. Real-time DPI measurements of mass decreases on the surface of a sensor chip modified with the dsDNA probe (*PC*) after adding the correct target *X* and different single-base change sequences at the 11th nucleotide position (the branch migration region). The labels "m," "d," and "i" represent mutation, deletion, and insertion, respectively.

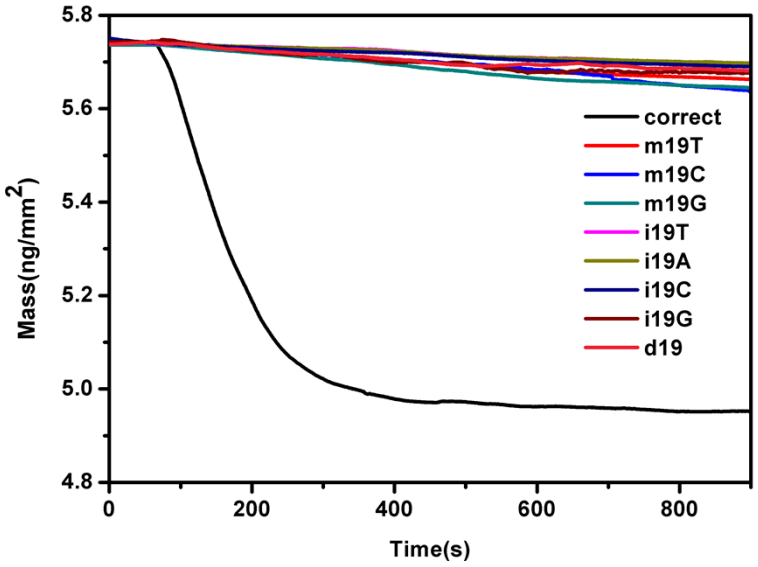

Figure S3. Real-time DPI measurements of mass decreases on the surface of a sensor chip modified with the dsDNA probe (*PC*) after adding the correct target *X* and

different single-base change sequences at the 19th nucleotide position (the toehold region). The labels "m," "d," and "i" represent mutation, deletion, and insertion, respectively.

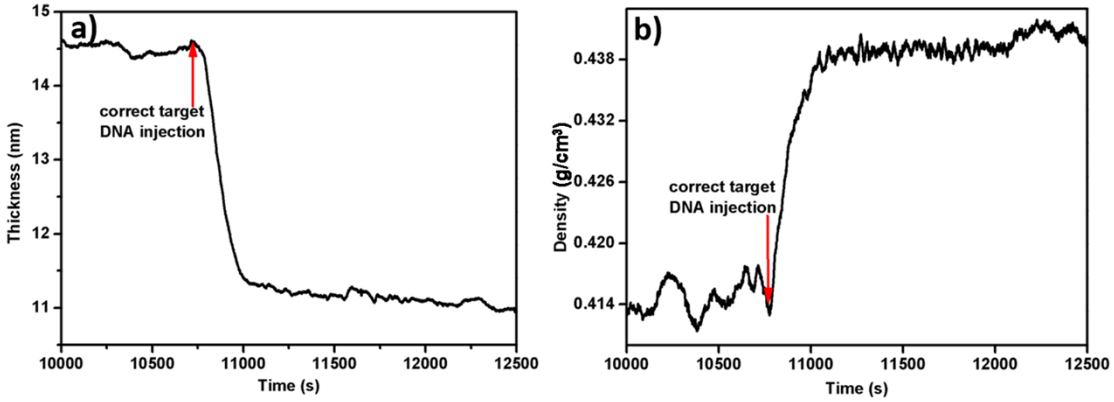

Figure S4. Real-time DPI measurements of (a) thickness and (b) density on the surface of a sensor chip modified with the dsDNA probe (*PC*) when the correct target *X* was added.

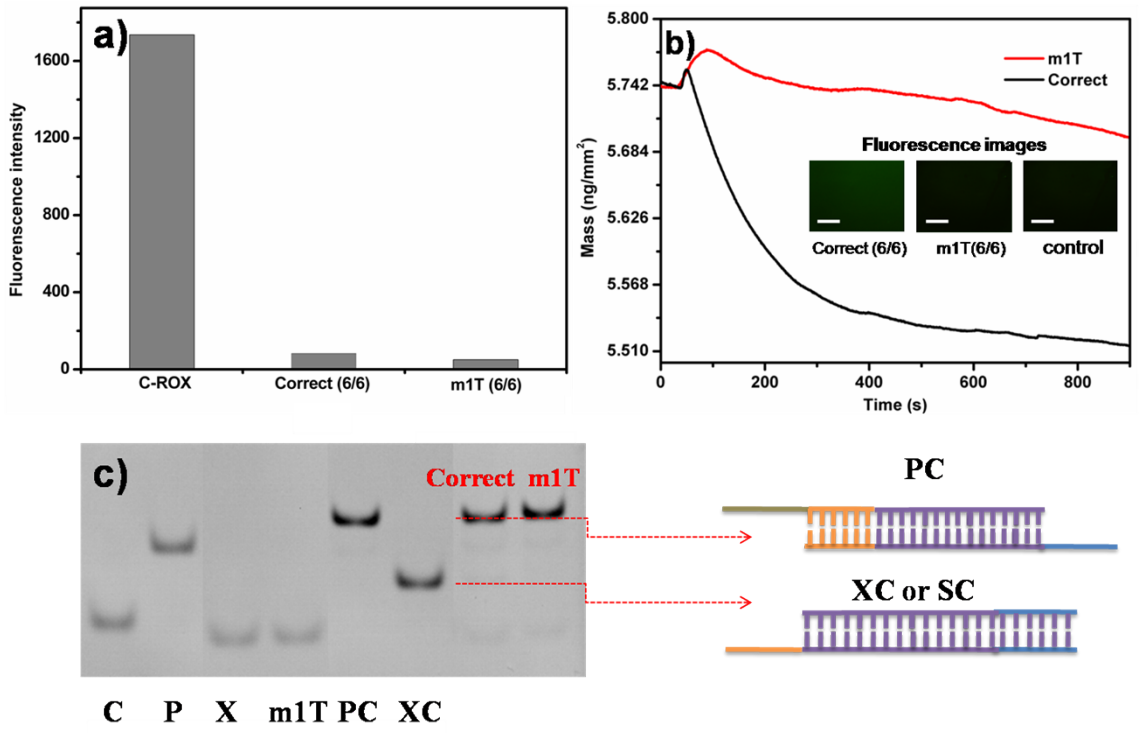

Figure S5. Toehold exchange of the 6/6 probe after adding the correct target (*X*) and

spurious target (*S*) in the solution and on the chip surface. (a) Fluorescence test of the toehold exchange of the 6/6 probe in the solution after adding *X* and *S* with a single-base mismatch at the 1st nucleotide position. The bar C-ROX is the fluorescence intensity of the pure single-strand *C-ROX* (100 nM). In a typical experiment, the probe *PC* was prepared with a 1.1:1 ratio of *P*-quencher to *C-ROX*. *X* or *S* was added to achieve the final concentrations of 200 nM for the target (*X* or *S*), 100 nM for *PC*, and 10 nM for *P*. (b) Real-time DPI measurements of mass decreases on the surface of a sensor chip modified with the 6/6 probe upon the addition of *X* and *S* with a single-base mismatch at the 1st nucleotide position (inset shows the fluorescence images of the chip surface after toehold exchange and addition of FAM-modified reporter). (c) PAGE analysis of toehold exchange of the 6/6 probe in the solution upon the addition of *X* and *S* with a single-base mismatch at the 1st nucleotide position. The probe *PC* was prepared with a 1.1:1 ratio of *P-tail* to *C*. *X* or *S* was added to achieve the final concentrations of 200 nM for the target (*X* or *S*), 100 nM for PC, and 10 nM for *P*. Toehold exchange proceeded at 20 °C for 1 h.

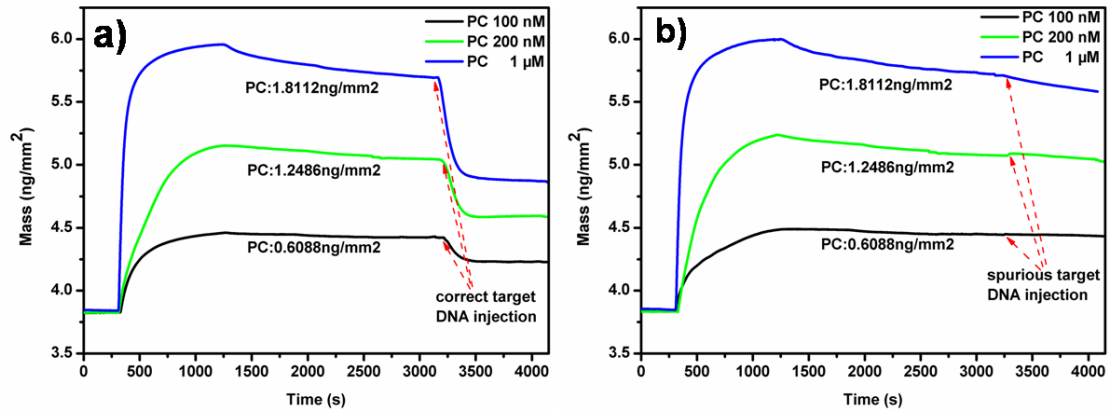

Figure S6. Real-time DPI measurements of surface mass decrease on the chip surface with different surface densities of immobilized dsDNA probe (*PC*) upon the addition of (a) correct target ssDNA and (b) spurious target ssDNA. In

a typical experiment, the dsDNA probe (*PC*) solutions with different concentrations (100 nM, 200 nM, and 1 μM) in TE/Mg^{2+} buffer were injected into both channels for 15 min at 10 μL min^{-1} to obtain a chip surface with different amounts of immobilized dsDNA probe (0.6088, 1.2468, and 1.8112 ng/mm^2). Then, solutions of the correct target (Correct) and spurious target (m6T) at a concentration of 1 μM were injected into two channels to investigate toehold exchange. The toehold exchange efficiencies after adding the correct target ssDNA (Correct) are 81.6% (blue curve), 75.2% (green curve), and 63.8% (black curve), whereas those after adding the spurious target ssDNA (m6T) are 11.9%, 9.3%, and 5.9%.

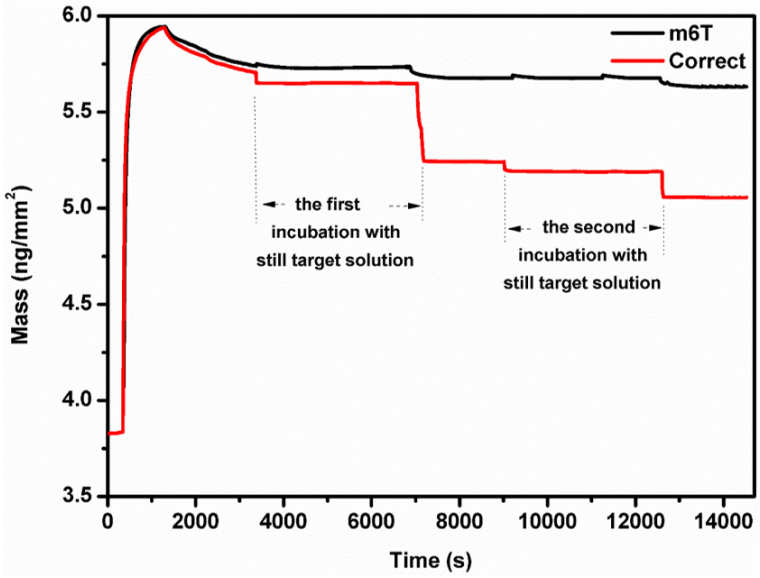

Figure S7. Real-time DPI measurements of surface mass decrease on the chip surface modified with the dsDNA probe (*PC*) after incubation with the "still" correct target (Correct) and the spurious target solution (m6T) for 1 h. After incubation, the chip surface was washed with TE/Mg^{2+} for 30 min.

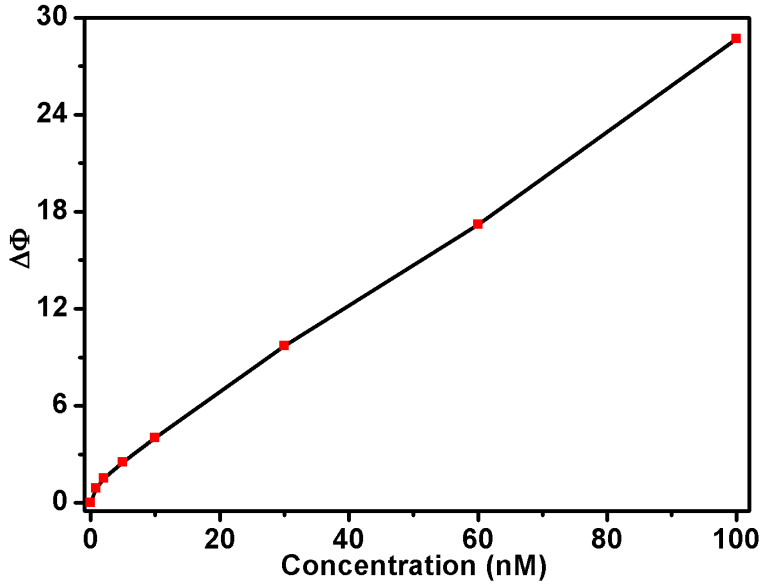

Figure S8. Reaction sensitivity on the chip surface to detect single-base changes. The difference in reaction efficiency between the correct and spurious targets is ($\Delta\emptyset$); the concentration of targets solution ranges from 0.8 nM to 100 nM. The detection limit is 0.8 nM.

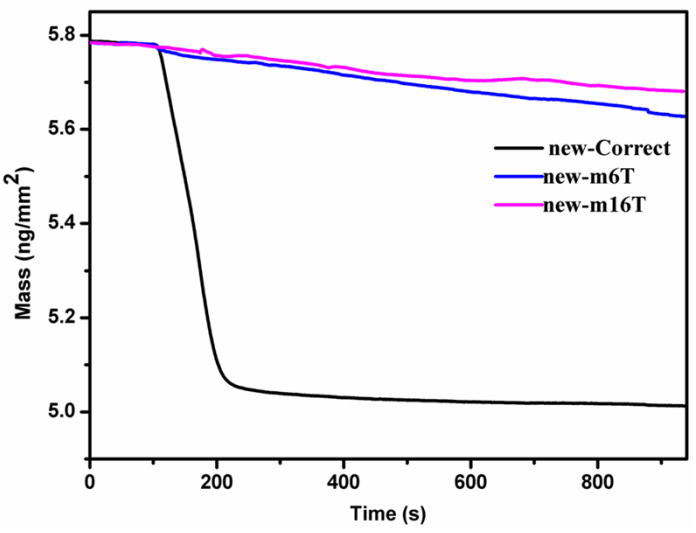

Figure S9. Real-time DPI measurements of surface mass decrease on the sensor chip surface modified with a new dsDNA probe (new-PC) after incubation with the correct target ssDNA (new-Correct). Different new spurious targets have a single-base mismatch at different positions (new-m6T and new-m16T). The

concentrations of the new correct and spurious targets are both 1 μM.

References:

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(4) Tumolo, T.; Angnes, L.; Baptista, M. S., *Anal. Biochem.* **2004**, *333*, 273-279.