Supporting Information for

Detection of Single-Nucleotide Polymorphisms Using an ON-OFF Switching of Regenerated Biosensor Based on Locked Nucleic Acid-Integrated and Toehold-Mediated Strand Displacement Reaction

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Table S1 DNA sequences used in this work.

Oligonucleotide name	Sequence (5' to 3') description [a]
Thiolated DNA probe (T-P)	SH-$(CH_2)_6$-TGAACAGCTTTGAGGTGC
Capture probe:	**CAAACAa**GCACCTCAAAGCTGTTC-methylene
LNA-integrated probe (MB-L)	blue
Capture probe:	**CAAACAA**GCACCTCAAAGCTGTTC-methylene
DNA probe (MB-D)	blue
Mutant-type target DNA probe: TM-34	GAACAGCTTTGAGGTGCTTGTTTGTGCCTGTCCT
Mutant-type target DNA probe: TM-44	GAACAGCTTTGAGGTGCTTGTTTGTGCCTGTCCT GGGAGAGACC
Mutant-type target DNA probe: TM-8	GAACAGCTTTGAGGTGCTTGTTTGT
Mutant-type target DNA probe: TM-7	GAACAGCTTTGAGGTGCTTGTTTG
Mutant -type target DNA probe: TM-6	GAACAGCTTTGAGGTGCTTGTTT
Mutant -type target DNA probe: TM-5	GAACAGCTTTGAGGTGCTTGTT
Mutant -type target DNA probe: TM-4	GAACAGCTTTGAGGTGCTTGT
Mutant A	GAACAGCTTTGAGGTGCATGTTTG
Mutant C	GAACAGCTTTGAGGTGCCTGTTTG
Random	TGGACGTAGTATTGGACGTAGTAT
Wild-type DNA probe: WT	GAACAGCTTTGAGGTGC*G*TGTTTG

[a] The underlined region of T-P is the recognition sequence for the capture probe. The bold letters in the capture probe represent toehold region. The lowercase letter "a" in MB-L represents LNA nucleotide and italicized letter "*G*" in WT represents mismatched nucleotide.

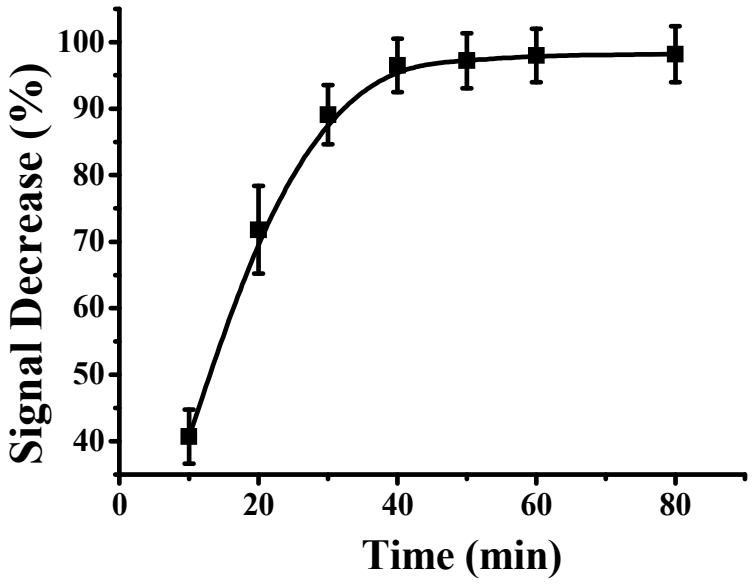

Figure S1. Relationship between the percentage change of ACV current ($I/I_{MB\text{-}L}$, where I is the peak current at various hybridization time points, and $I_{MB\text{-}L}$ is the peak current at equilibration time) and the various hybridization time (10, 20, 30, 40, 50, 60, and 80 min) of T-P/MB-L hybrids. Each concentration of DNA probe used in the assay is 100 nM. Error bars indicate standard deviation (n = 3).

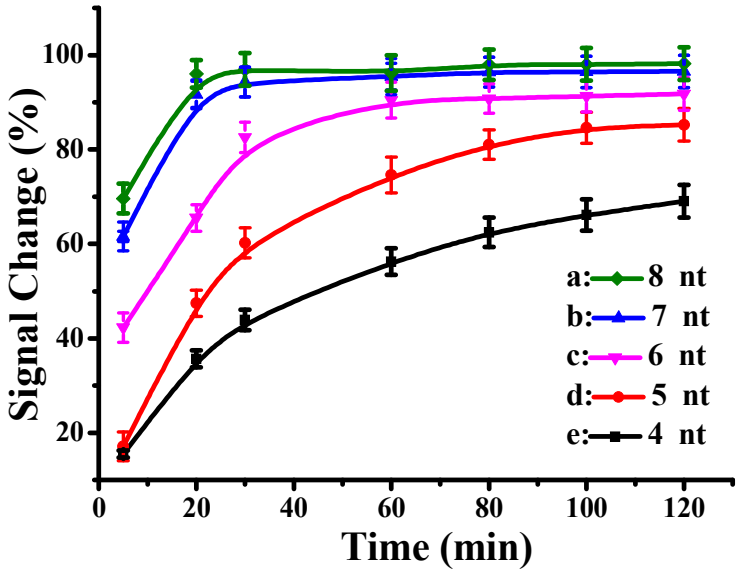

Figure S2. Percentage change of ACV current (($I_{MB\text{-}L} - I_{TM\text{-}4/5/6/7}$)/$I_{MB\text{-}L}$, where $I_{MB\text{-}L}$ and $I_{TM\text{-}4/5/6/7}$ are the peak current before and after addition of TM-4, TM-5, TM-6, TM-7, and TM-8 respectively.) responses to the various hybridization time (5, 20, 30, 60, 80, 100, and 120 min) based on toehold-mediated strand displacement reaction to mutant-type target DNA with different toehold lengths (a: 8 nt, b: 7 nt, c: 6 nt, d: 5 nt, e: 4 nt). Each concentration of DNA probe used in the assay is 100 nM. Error bars indicates standard deviation (n = 3).

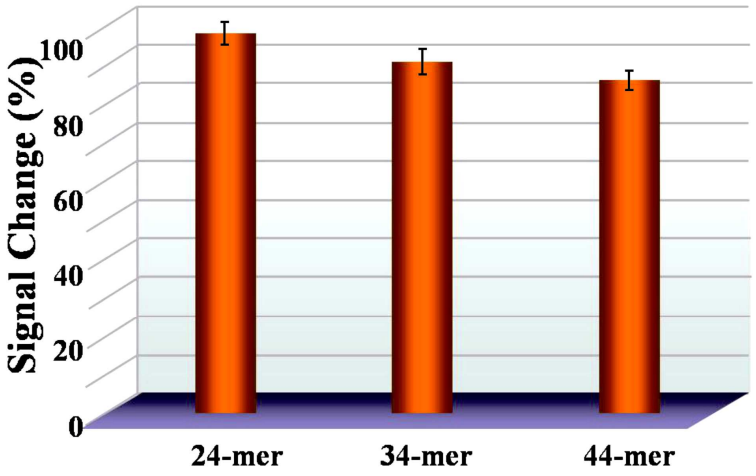

Figure S3. Percentage change of ACV current (($I_{MB\text{-}L} - I_{24\text{-}mer/\ 34\text{-}mer/\ 44\text{-}mer}$)/$I_{MB\text{-}L}$, where $I_{MB\text{-}L}$ and $I_{24\text{-}mer/\ 34\text{-}mer/\ 44\text{-}mer}$ are the peak current before and after addition of 24-mer/ 34-mer/ 44-mer DNA sequences, respectively.) responses to the various lengths of DNA sequences. Each concentration of DNA probe used in the assay is 100 nM. The data are collected from at least three independent sets of experiments.

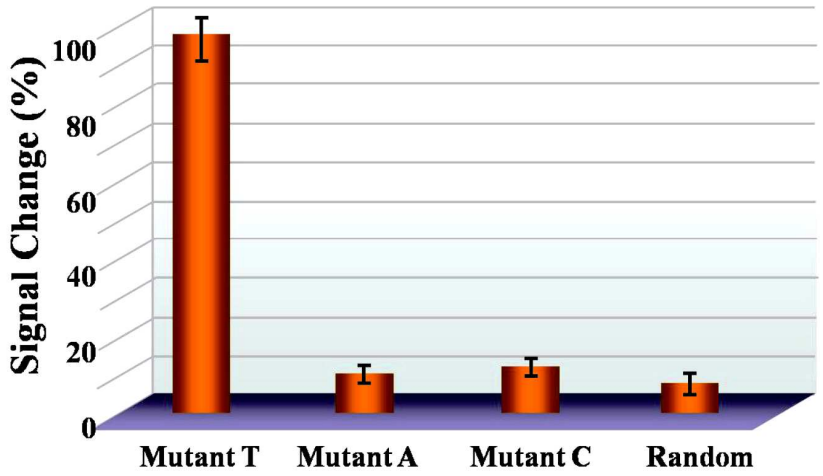

Figure S4. Percentage change of ACV current (($I_{\text{MB-L}} - I_{\text{Mutant T/ Mutant A/ Mutant C /Random}}$)/$I_{\text{MB-L}}$, where $I_{\text{MB-L}}$ and $I_{\text{Mutant T/ Mutant A/ Mutant C/Random}}$ are the peak current before and after addition of mutant T, mutant A, mutant C, and random, respectively.) responses to the various mismatched DNA probes. Each concentration of DNA probe used in the assay is 100 nM. The data are collected from at least three independent sets of experiments.

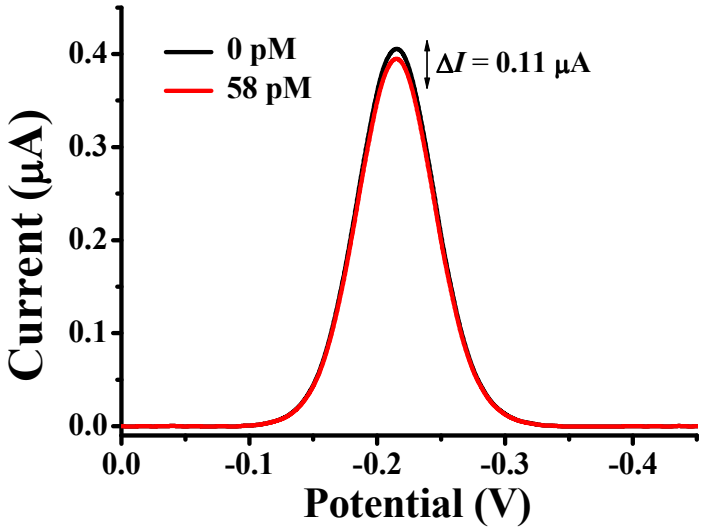

Figure S5. Alternating current voltammograms of the sensor in the presence of 0 and 58 pM TM-7 target. All measurements are performed in PB solution (100 mM phosphate, pH 7.4). The data shown are average measurements from three different electrodes.

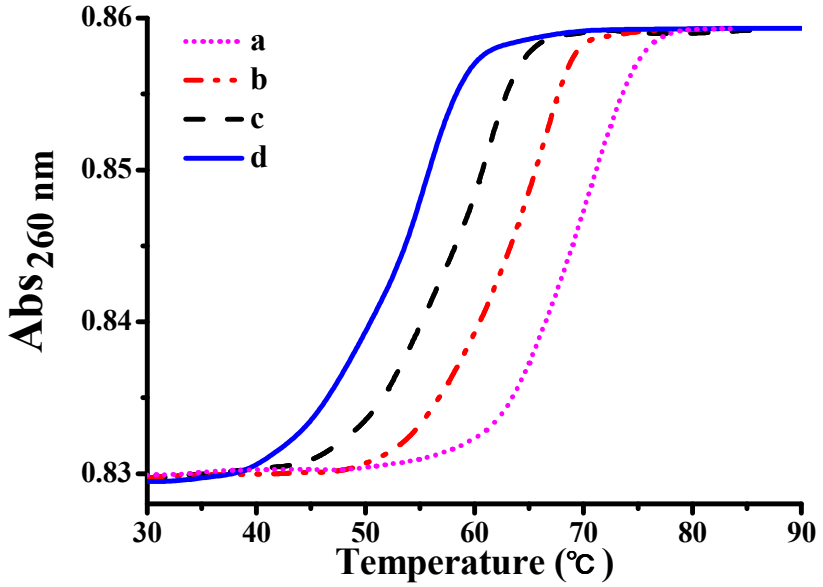

Figure S6. UV melting measurements of MB-L with perfectly matched TM-7 probe (a) and single base mismatched WT probe (d), and those of MB-D with perfectly matched TM-7 probe (b) and single base mismatched WT probe (c), respectively. Experiment is conducted in 20 mM Tris-HCl solution containing 100 mM NaCl (pH 7.4).