Supporting Information

Assembly of Multiple DNA Components through Target Binding toward Homogeneous, Isothermally Amplified, and Specific Detection of Proteins

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Table S1. Sequences and modifications of oligonucleotides used in this study

Name	Sequence (5'-3')
MB1	**/56-FAM/ CTA GCA GTC TCT AA TA CCT CAG CGC TAG/3IABkFQ/**
MB2	/56-FAM/ CGA GCA GTC TCT AA CCT CAG CGC TCG/3IABkFQ/
T-DNA-B6	/5BioTEG/TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT ACA CCA TTA GAG AC
T-DNA-B7	**/5BioTEG/TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT G ACA CCA TTA GAG AC**
T-DNA-B8	/5BioTEG/TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT CAG ACA CCA TTA GAG AC
T-DNA-B9	/5BioTEG/TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT AAG ACA CCA TTA GAG ACT
A-DNA-A7	**G CTG AGG TGG TGT C TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT/3BioTEG/**
A-DNA-A8	CG CTG AGG TGG TGT C TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT/3BioTEG/
A-DNA-A9	GCG CTG AGG TGG TGT C TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT/3BioTEG/
Blocking DNA-10	**CTC TAA TGG T**
Blocking DNA-11	CTC TAA TGG TG
Blocking DNA-12	CTC TAA TGG TGT

Ta**ble S2.** Free energy (ΔG) values of tested complementary sequences (A to A*, B to B* and T to T*) in this study. The ΔG values were estimated by UNAFold under a condition of 50mM NaCl and 10 mM $MgCl_2$.

Length of A to A*	ΔG (kcal/mol)
7 bases	-6.8
8 bases	-9.0
9 bases	-11.0

Length of B to B*	ΔG (kcal/mol)
6 bases	-5.8
7 bases	-7.3
8 bases	-8.3
9 bases	-9.2

Length of T to T*	ΔG (kcal/mol)
8 bases	-5.4

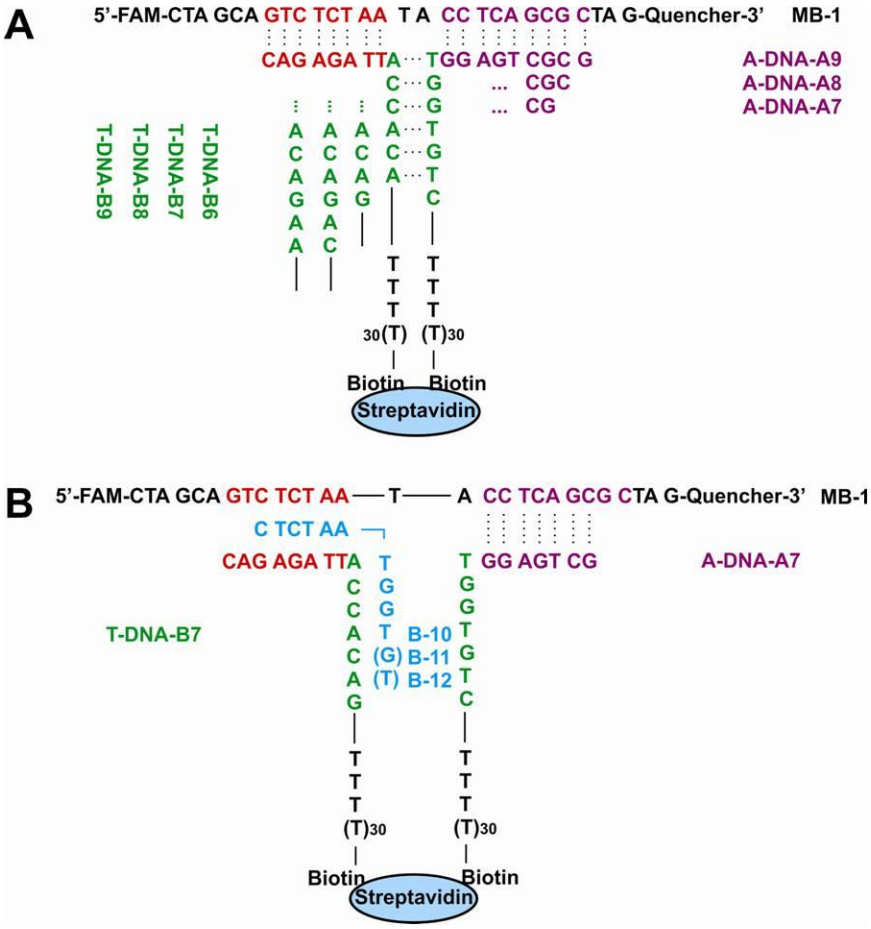

Figure S1. Schematic showing different designs of T-DNA, A-DNA, and MB (A), and blocking DNA (B).

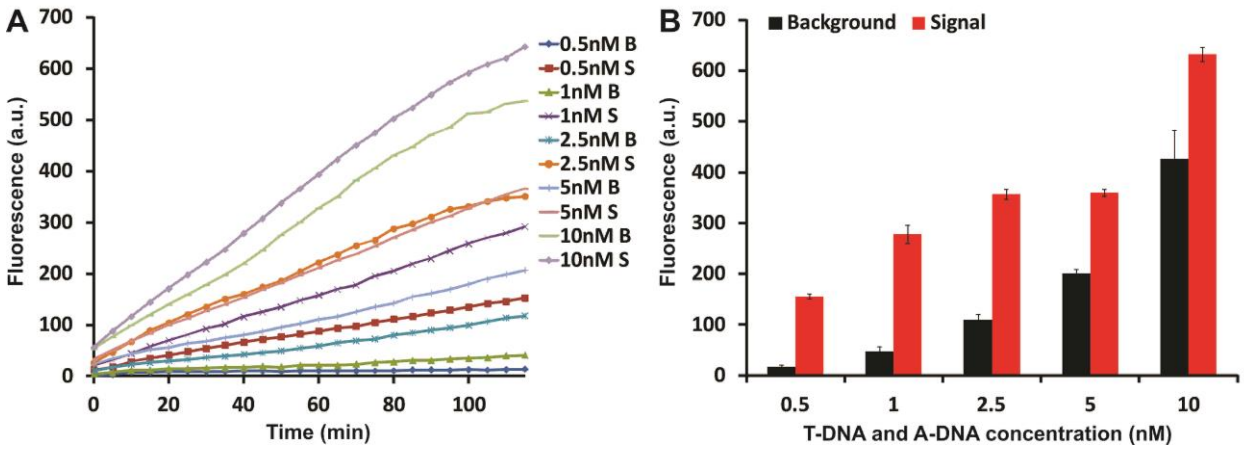

Figure S2. Impact of T-DNA and A-DNA concentration on signal and background. (A) Amplification curves from analysis of 200 pM streptavidin using different concentrations of T-DNA and A-DNA. (B) Signal and background values of endpoints.

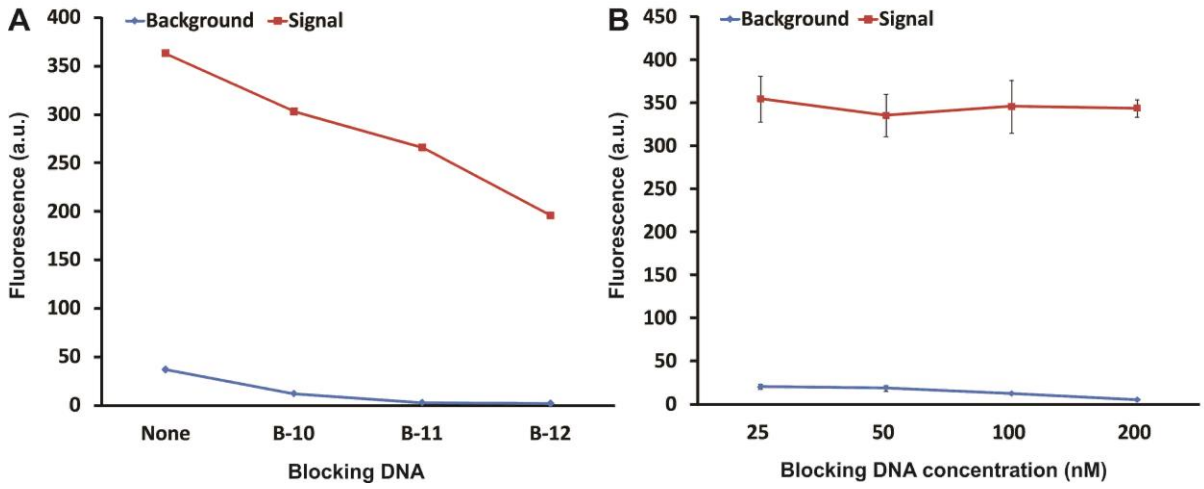

Figure S3. Impact of blocking DNA with varying lengths on the signal and background. (A) Comparison of different blocking DNA for reducing background and signal. Blocking DNA having 10 bases resulted in the largest signal to background ratio. 50 nM of blocking DNA was used. (B) The impact of concentration of blocking DNA (10 bases) on signal and background. The background decreased with increasing concentration of blocking DNA.

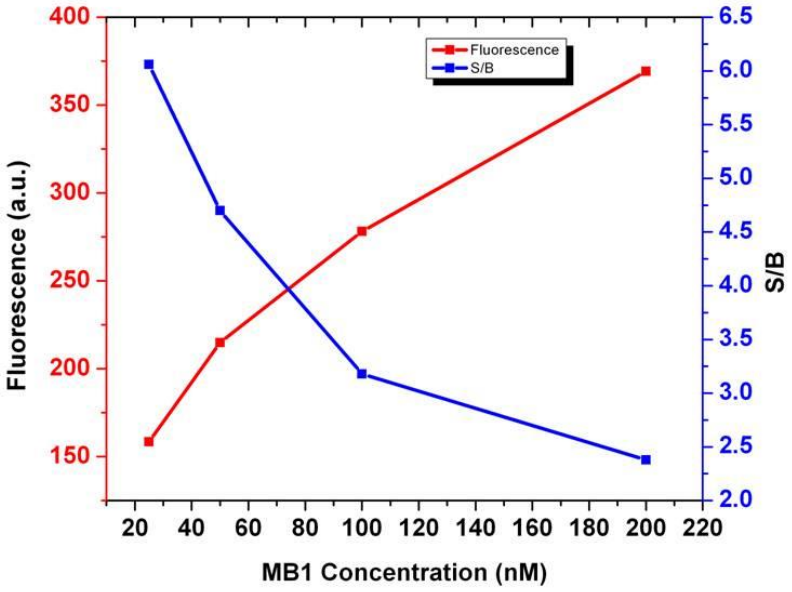

Figure S4. Impact of MB concentration on the signal and background. 200 pM streptavidin was analyzed by using different concentrations of MB1. Fluorescence signal intensity increased with increasing MB1 concentration, while signal to background ratio decreased with increasing MB1 concentration because higher MB1 concentration showed higher background fluorescence from incomplete quenching. 100 nM MB1 showed satisfactory results in both fluorescence signal intensity and signal to background ratio.

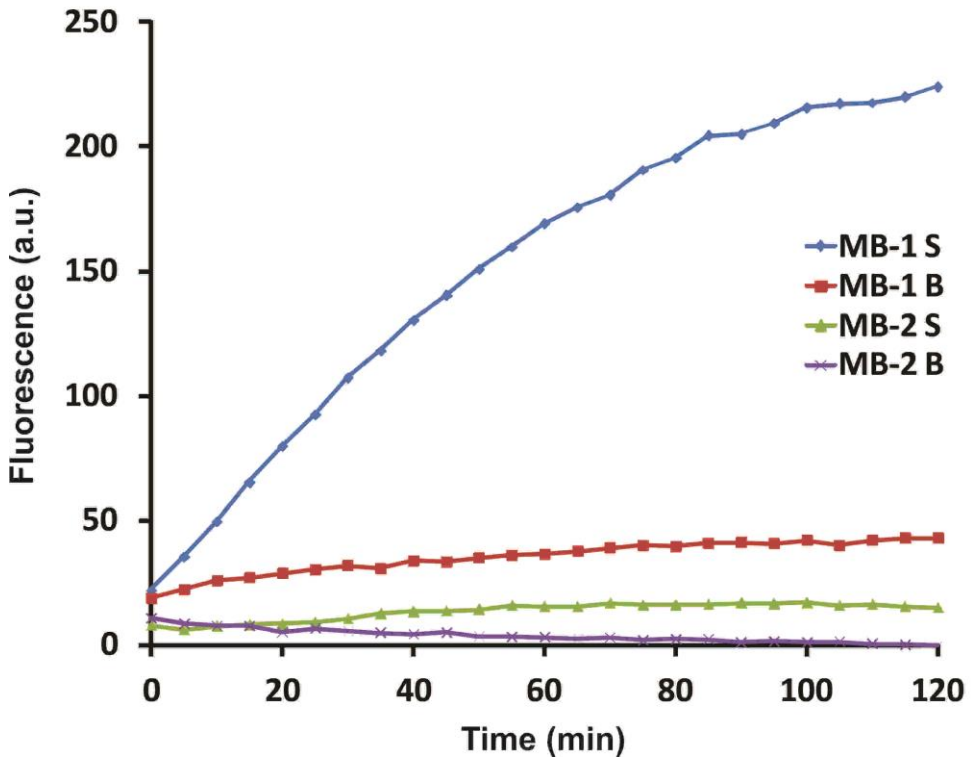

Figure S5. Comparison of performances of MB1 and MB2 for analysis of 200 pM streptavidin using T-DNA and A-DNA containing B of 8 bases and A of 8 bases, respectively. "S" means signal and "B" means background.

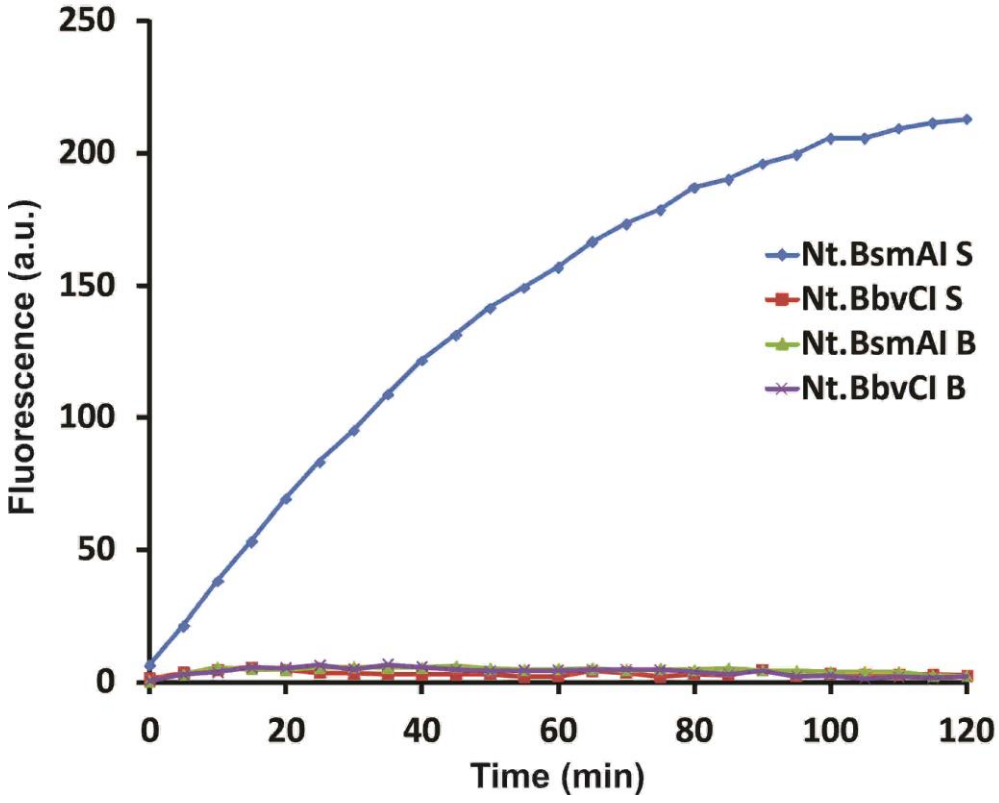

Figure S6. Comparison of performances of two NEases, Nt.BsmAI and Nt.BbvCI for analysis of 200 pM streptavidin. No amplification was observed when Nt.BbvCI was used. "S" means signal and "B" means background.

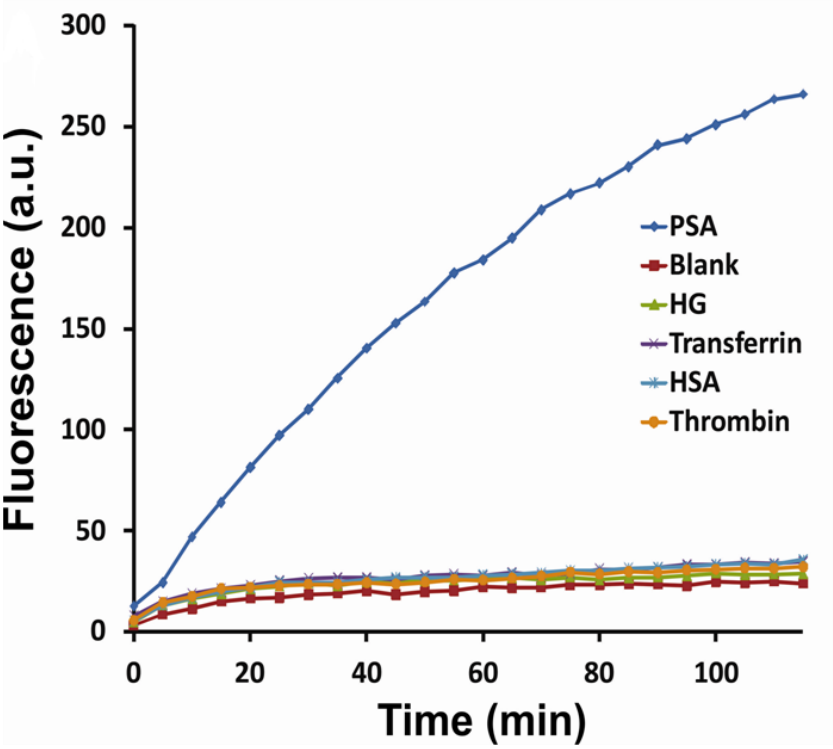

Figure S7. Examining **s**pecificity of the assay by applying it to detection of four other proteins. HG means human immunoglobulin, and HSA means human serum albumin. The concentration of PSA was 1 nM, and concentrations of other four proteins were all 10 nM.