Electronic Supplementary Information

Amplified Binding-Induced Homogeneous Assay through Catalytic Cycling of Analytes for Ultrasensitive Protein Detection

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EXPERIMENTAL SECTION

Apparatus. Fluorescence intensity of the testing solutions was measured with a multimode microplate reader (DX800, Beckman Coulter) at room temperature. The fluorescence intensity at 535nm was used to evaluate the performance of the proposed assay.

Materials and Reagents. All DNA oligonucleotides were purchased, modified, and purified from Integrated DNA Technologies (IDT, Coralville, IA). The DNA sequences are listed in Table 1 and Table S1 of the Electronic Supplementary Information. Biotin, streptavidin, bovine serum albumin (BSA), prostate-specific antigen (PSA), and human serum were purchased from Sigma-Aldrich (Oakville, ON). Biotinylated polyclonal anti-PSA antibody was purchased from R&D Systems (Minneapolis, MN). Nicking endonucleases (NEases), Nt.BstNBI, and 10× NEB buffer (500mM potassium acetate, 200 mM Tris-acetate, 100 mM magnesium acetate, 10 mM dithiothreitol, pH 7.9) were obtained from New England BioLabs (Whitby, ON). Phosphate-buffered saline (1× PBS) (137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4) was diluted with deionized water from 10× PBS buffer (Fisher Scientific, Nepean, ON). All other reagents were of analytical grade.

Table S1. Blocking DNA sequences used in this work.

Oligos	Sequence 5'→3'
Blocking DNA-1	C GCG GAC TCT GAA AAA A
Blocking DNA-2	AC GCG GAC TCT GAA AAA A
Blocking DNA-3	CAC GCG GAC TCT GAA AAA A
Blocking DNA-4	G CAC GCG GAC TCT GAA AAA A
Blocking DNA-5	GG CAC GCG GAC TCT GAA AAA A
Blocking DNA-6	TGG CAC GCG GAC TCT GAA AAA A
Blocking DNA-7	C TGG CAC GCG GAC TCT GAA AAA A
Blocking DNA-8	AC TGG CAC GCG GAC TCT GAA AAA A
Blocking DNA-9	AAC TGG CAC GCG GAC TCT GAA AAA A
Blocking DNA-10	AAC TGG CAC GCG GAC TCT GAA AAA AGC CCC G

Blocking DNA-11	CTG GCA CGC GGA CTC TGA AAA AAG CCC CG

Table S2 Melting temperature (Tm) of the designed duplexes.

Duplex	Tm (ºC)
Hairpin	72
Probe-B: Hairpin	52.7
Probe-F: Hairpin	17.6
Probe-F: Hairpin after binding induced-assembly	48.4

Melting temperature (Tm) of the designed duplexes was estimated by using Oligo Analyzer under conditions of 100 mM NaCl, and 10 mM $MgCl_2$.

Experimental Optimization of the Assay Using Streptavidin as a Target. Output DNA was designed as a fully complementary target to FAM-labelled ssDNA (F) to optimize the key parameters of the assay, including the blocking DNA, the concentration of blocking DNA, and the concentration of graphene oxide (GO). To obtain the reliable optimized data, all of the parameters in this assay, including the probe-F, probe-B, hairpin structure, reaction buffers and components, are still fixed except nicking enzyme. Optimization of a specific parameter was conducted by analysis, including sample and blank solutions. Other conditions were fixed except the parameter being optimized. Sample solutions were prepared to contain 100 pM Streptavidin, 100 nM Hairpin structure, 2 nM Probe-B, 2 nM Probe-F, blocking DNA, 10 units T4 DNA ligase and 0.1% BSA in 100 μLl of 1× NEB buffer (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol, pH 7.9). Blank solutions contained all of other ingredients except Streptavidin. The sample and blank solutions were placed in 55ºC for amplification for 2 h and cooling down to room temperature. Then the FAM-labelled ssDNA (F) was added into the sample and blank solutions, and final concentration was 100 nM. After 10 min incubation, GO was added into each solutions. All of the solutions were then transfered into a 96-well plate which was loaded into multimode microplate reader. In each analysis, all of the testing solutions were duplicated. The fluorescence intensity of the sample solutions and the blank solutions was used to calculate the signal-to-background ratio.

Feasibility Test and Detection of Streptavidin. Detection of streptavidin in sample and blank solutions was carried out. Sample solutions contained 100 pM streptavidin, 100 nM hairpin structure, 2 nM Probe-B, 2 nM Probe-F,200 nM blocking DNA , 10 units T4 DNA ligase, 10 units nicking enzyme and 0.1% BSA in 100 μL of 1× NEB buffer. Blank solutions contained all of other ingredients except 100 pM streptavidin. The sample and blank solutions were placed in 65ºC for amplification for 2 h and cooling down to room temperature. Then the 100 nM FAM-labelled ssDNA (F) was added into the sample and blank solutions. After 10 min incubation, 60 μg/mL GO

was added into each solution. All of the solutions were recorded by multimode microplate reader. In each analysis, all of the solutions were duplicated.

Optimization of Blocking DNA. Before output DNA being released, we design 11 blocking DNA with varied length to partially bind to the Output DNA on the hairpin against FAM-labelled ssDNA. We compared 11 blocking DNA (from Blocking-1 to Blocking-11), each contained sample and blank solution with 100 nM blocking DNA. 100 pM streptavidin as detection target for optimization. Each analysis incubated for 10 min before being loaded into the multimode microplate reader.

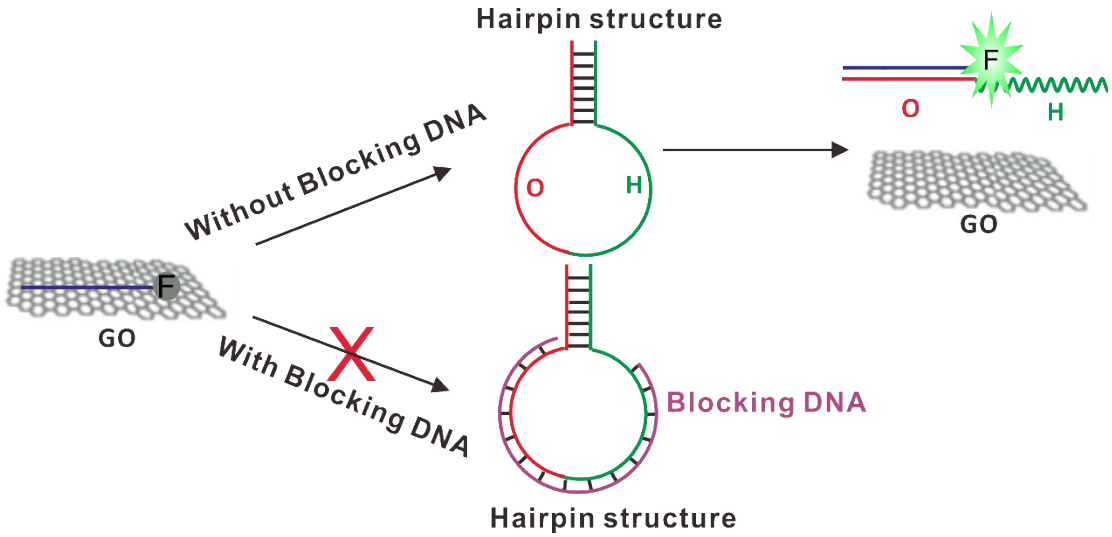

Figure S1 Schematic showing principle of blocking DNA (Purple).

Table S3. The melting temperatures (Tm) and Free energy (ΔG) values of tested

Complementary sequences	Complementary Length (bp)	ΔG (kcal/mole)	Tm (°C)
Blocking DNA-8 (B-8) and intact hairpin structure	24	-50.71	72
Blocking DNA-8 (B-8) and output DNA	7	-12.89	19.2
FAM-labelled ssDNA and intact hairpin structure	14	-31.91	61.4
FAM-labelled ssDNA and output DNA	14	-31.91	61.4

Complementary sequences between blocking DNA-8 and intact hairpin structure or output DNA, FAM-labelled ssDNA and intact hairpin structure or output DNA in this study. The Tm and ΔG values were estimated by using Oligo Analyzer (free software from IDT) under a condition of 50 mM NaCl and 10 mM $MgCl_2$.

Optimization of Different Blocking DNA. The impact of different blocking DNA

with varied length on signal and background. 100 pM streptavidin as detection target for optimization.

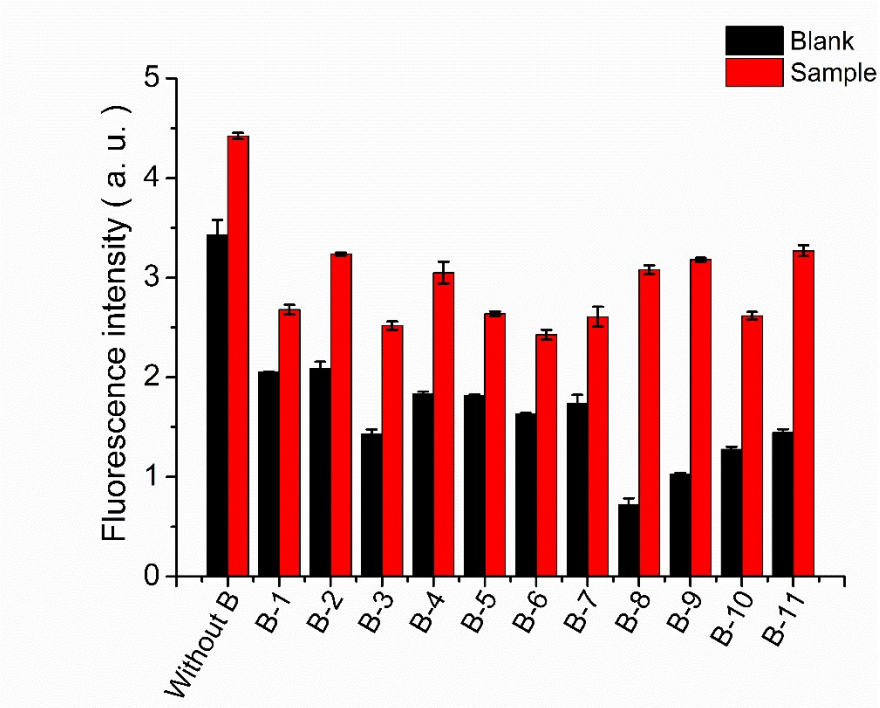

Figure S2. Impact of blocking DNA with varied lengths. Comparison of different blocking DNA for reducing background and signal. Blocking DNA-8 with 10 bases gives best signal to background ratio.

Optimization of Concentration of Blocking DNA-8. The impact of concentration of blocking DNA (10 bases) on signal and background. The background decreased with increasing concentration of blocking DNA. 100 pM streptavidin as detection target for optimization. Each analysis was done after incubation for 10 min before being loaded into the multimode microplate reader.

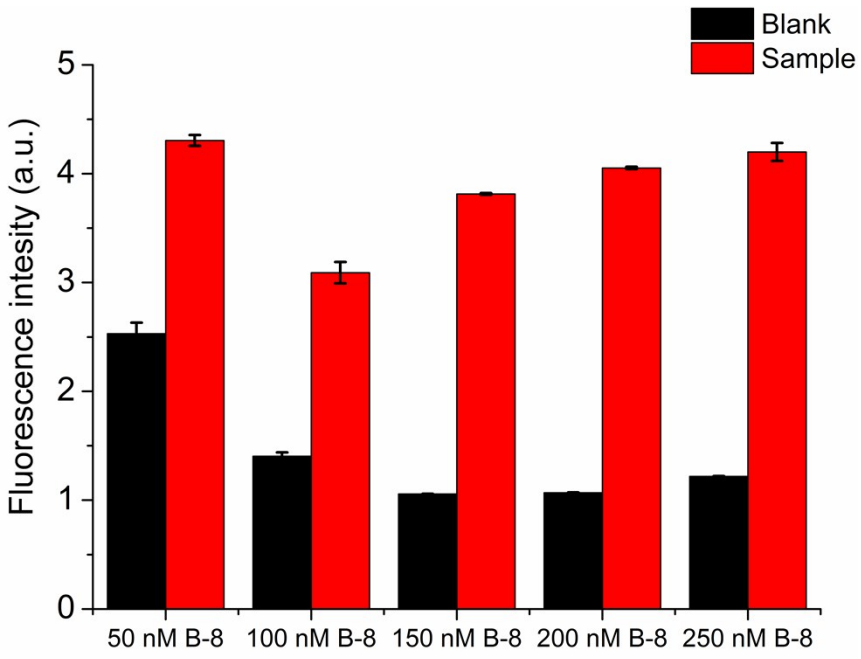

Figure S3. Impact of blocking DNA-8 with different concentration. 200 nM blocking DNA-8 give rise to best signal to background ratio.

Optimization of Concentration of graphene oxide (GO). The impact of concentration of GO on signal and background. 100 pM streptavidin as detection target for optimization. Each analysis was done after incubation for 10 min before being loaded into the multimode microplate reader.

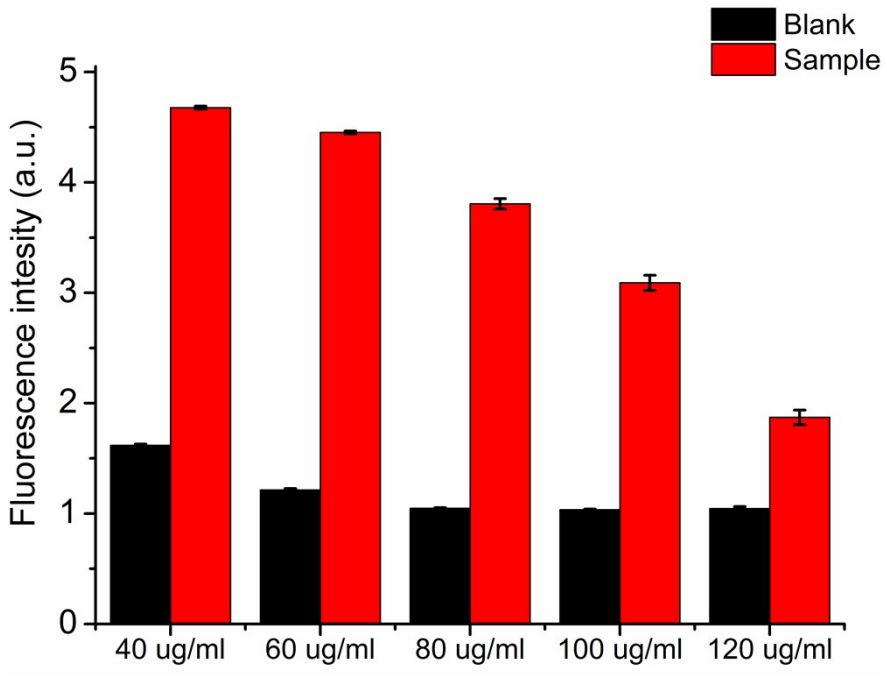

Figure S4. Impact of GO concentration on the signal and background. 60 μg/mL GO showed satisfactory results in both fluorescence signal intensity and signal to background ratio.

Detection of Prostate-Specific Antigen. To detect PSA, we first took advantage of biotin-streptavidin interaction to construct Probe-B and Probe-F linked with biotinylated polyclonal anti-PSA antibodies. Specifically, 100 μL of 800 nM biotinylated Probe-B and Probe-F was mixed with 100 μL of 800 nM streptavidin in 1X PBS buffer. After incubation at room temperature for 1 h, 200 μL of 400 nM biotinylated anti-PSA antibodies was added to each mixture. The mixtures were incubated for another 30 min for conjugation between Probe-B, Probe-F and antibody. The conjugated DNA probes were further diluted to 100 nM in PBS buffer containing 0.1% BSA and 10 μM biotin. The excess amount of biotin was employed to occupy the free biotin-binding sites of streptavidin. The probe solutions were stored at 4°C prior to use. Serial concentrations of PSA were diluted in 100 μL of 1×NEB buffer or of 5% human serum in 1× NEB buffer. Each contained 100 nM nairpin structure, 2 nM Probe-B, 2 nM Probe-F, 200 nM blocking DNA, 10 units T4 DNA ligase, 10 units nicking enzyme and 0.1% BSA. Prior to enzymatic reaction, all of solutions were placed at room temperature to assist binding of antibodies to PSA molecules. The fluorescence values of the 2 h amplification end points were used to establish calibration curves.