

## Electronic Supplementary Information

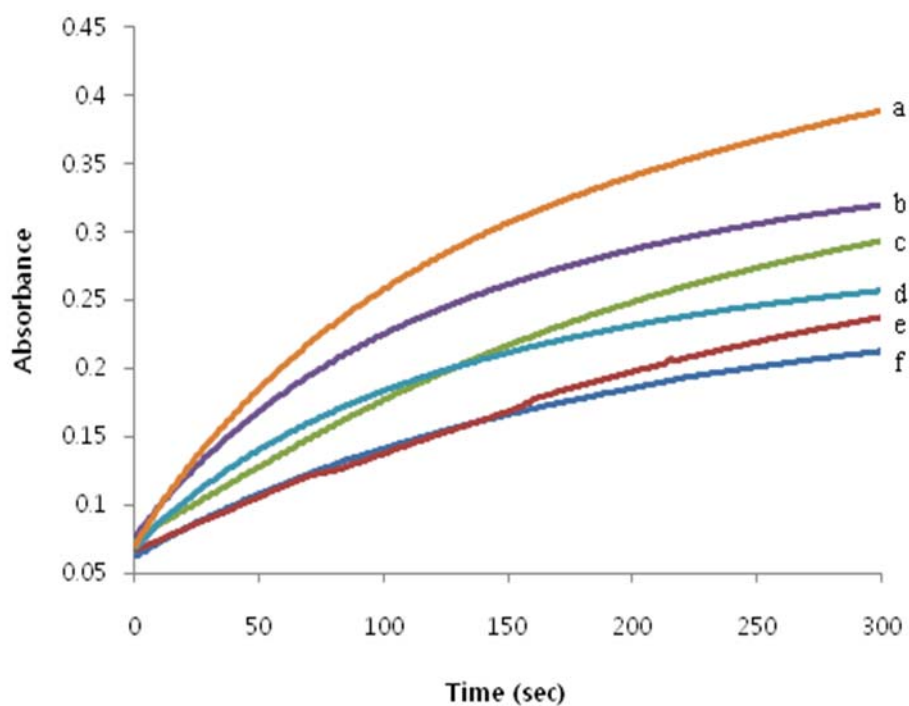
# **An Ultrasensitive Peroxidase DNzyme-Associated Aptasensor that Utilize a Target-Triggered Enzymatic Signal Amplification Strategy**

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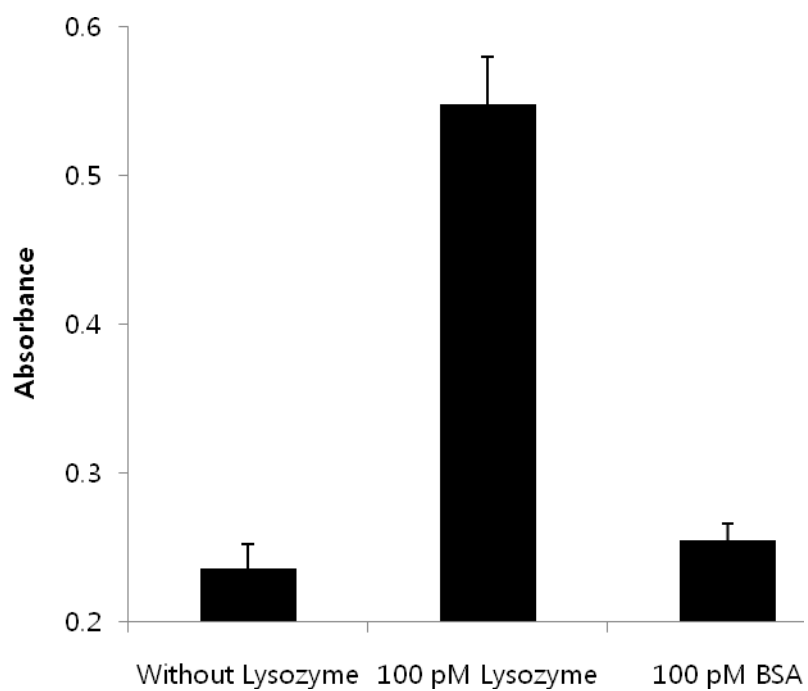
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**Fig. S1** Absorbance at 415 nm at fixed time intervals of 5 min resulting from the ABTS oxidation in the presence of lysozyme at different concentrations of (a) 100 nM, (b) 10 nM, (c) 1 nM, (d) 100 pM, (e) 10 pM, (f) 0 M



**Fig. S2** Absorption intensity at 415 nm obtained from the oxidation of ABTS for the analysis of negative control without lysozyme, 100 pM lysozyme, and 100 pM BSA after a fixed time interval of 5 min. Data are averages of three independent experiments.



## EXPERIMENTAL SECTION

### Materials and oligonucleotides

All chemicals were purchased from Sigma-Aldrich and used as supplied. A hemin stock solution (5 mM) was prepared in dimethyl sulfoxide (DMSO) and stored in the dark at -20 °C. The deoxynucleotide mixture (dNTPs), NEB buffer 2 solution, Klenow fragment exo-, and Nt. AlwI was purchased from New England Biolabs, Inc. (Beverly, MA, USA) and used without further purification. Doubly distilled water was used in all the experiments. All DNA oligonucleotides were synthesized and HPLC purified by using a Genotech Co. (South Korea) system. The sequences of DNAzymeMB, blocker DNA and primer used in this study are listed in Table 1.

**Table 1.** Sequences of DNAzymeMB, blocker DNA, and primer.

Name	Sequence
DNAzymeMB	5'- <b>AGGGACGGG</b> <i>CTAAGTAACTGTGGAGGGT</i> -3'
Blocker	5'- <b>ATCTACGAATTCATCAGGGCTAAAGAGTGCAGAGTTA</b> <b>CTTAGCCCGTGA</b> -3'
Primer	5'-TTGGAT <b>CACG</b> -3'

The bold letters in the DNAzymeMB sequence represents the stem portion. The underlined region of the Blocker is the sequence that is complementary to the underlined sequence of the DNAzymeMB. The bold letters in the Blocker sequence correspond to the aptamer region. The bold letters of primer correspond to the region that leads to annealing to the sequence in italic letters of the Blocker. The underlined region of the primer is the recognition sequence for nicking endonuclease Nt. AlwI.

### Recognition of target protein by using the inactive DNAzymeMB/blocker hybrid

One microliter of 50 μM DNAzymeMB and 1 μL of 50 μM blocker DNA were mixed in NEBuffer 2. The solution was treated at 94 °C for 4 min and cooled to room temperature. Then 1 μL of a solution of the sample was added to yield a total volume of 20 μL. After 30 min incubation at room temperature, 1 μL of 25 μM hemin and the detection buffer were added to make a total volume of 50 μL (25 mM HEPES, 20 mM KCl, 200 mM NaCl, 0.05% Triton X-100 and 1% DMSO; pH 7.4). A colorimetric measurement was then made.

### **Enzyme mediated, amplified detection of target proteins**

One microliter of 50  $\mu\text{M}$  DNAzymeMB/blocker hybrids, 1  $\mu\text{L}$  of 50  $\mu\text{M}$  primer, 1  $\mu\text{L}$  of Klenow fragment exo- (0.4 units), 1  $\mu\text{L}$  of Nt. AlwI (0.4 units), 2  $\mu\text{L}$  of dNTPs (0.2 mM), and 2  $\mu\text{L}$  of NEBuffer 2 (10 $\times$ ), (50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, pH 7.9) were added. The mixture was heated at 37 °C for 90 min followed by cooling to 4 °C. To the solution was added 1  $\mu\text{L}$  of a 25  $\mu\text{M}$  solution of hemin in the detection buffer to bring the total volume to 50  $\mu\text{L}$  (25 mM HEPES, 20 mM KCl, 200 mM NaCl, 0.05% Triton X-100 and 1% DMSO; pH 7.4). A colorimetric measurement was then made.

### **Colorimetric measurement**

For the colorimetric measurement, the ABTS/H<sub>2</sub>O<sub>2</sub> substrate was added to a vial containing the sample, yielding a total volume of 100  $\mu\text{L}$  (ABTS ( $7.2 \times 10^{-4}$  M) and H<sub>2</sub>O<sub>2</sub> ( $4.4 \times 10^{-5}$  M)). The absorbance at  $\lambda=415$  nm was determined by using a Cary 100 UV–Visible spectrophotometer (Varian, Palo Alto, CA).