Electronic Supplementary Materials (ESI)

Design and Optimization of an Ultra-Sensitive Hairpin DNA aptasensor for Salmonella Detection

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1. Binding affinity of EAD2-hemin complex

2 µM hemin was prepared in 50 mM tris-HCl (pH 7.4), 100 mM NaCl, 5 mM KCl and 1 mM MgCl$_2$. EAD2 was dissolved in same buffer. Hemin was mixed with various concentration of EAD2 and stood for at least an hour, and the dissociation constant (K_d) for the DNA-hemin complexes was determined by plotting the absorbance changes of hemin at 404nm against DNA concentrations (0 -10 µM). The K_d for EAD2 was determined to be
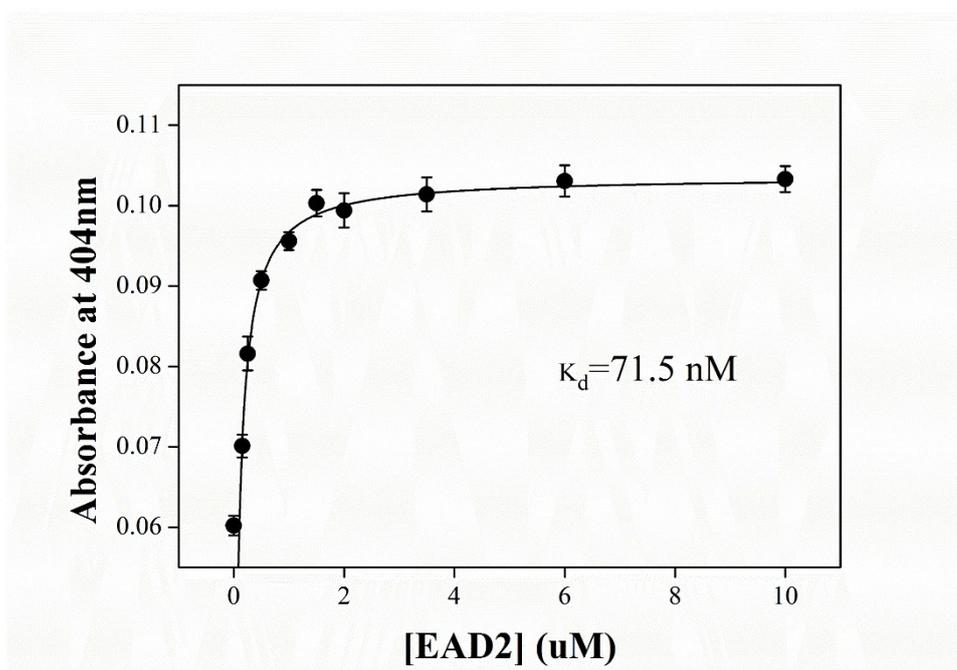

Figure S1. A plot for obtaining the dissociation constant for EAD2-hemin complex
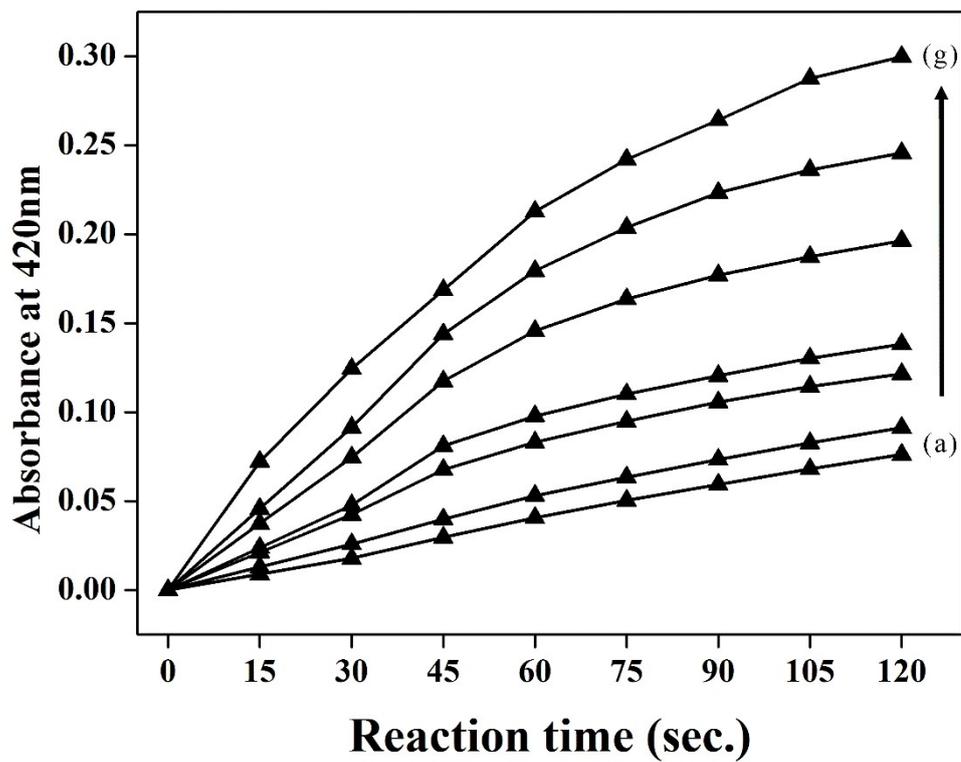
71.5 nM.

2. Kinetics of SHDs

Figure S2.1. Time-dependent Absorbance change of SHD$_4$ with different concentration of *S.* Typhimurium. Blank (a) 1x10^7 CFU/mL (b) 2x10^7 CFU/mL (c) 3.8x10^7 CFU/mL (d) 7x10^7 CFU/mL (e) 1x10^8 CFU/mL (f) 1.5x10^8 CFU/mL (g). [SHD$_4$]= 100nM, [hemin]= 300nM
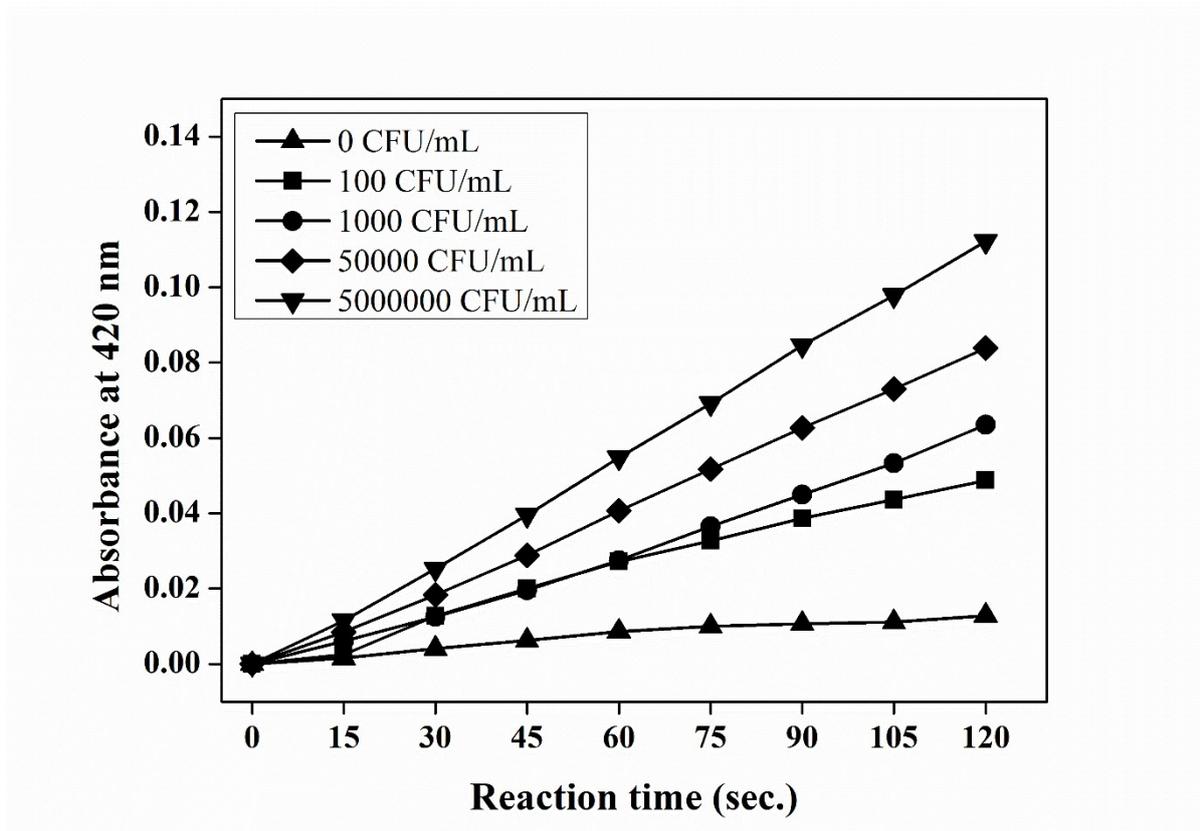
Figure S2.2. Time-dependent Absorbance change of *hem*-SHD$_2$ with different concentration of *S.* Typhimurium. [*hem*-SHD$_2$]= 200nM

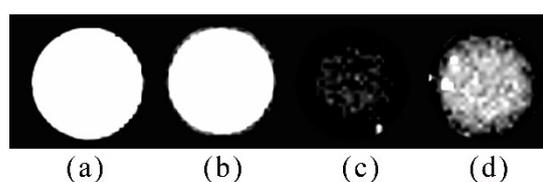## 3. CL inhibition of *p*-coumaric acid

(a) (b) (c) (d)

Figure S3. An accumulated CL image of reaction of 1 µM hemin mixture with 2mM of luminol and H$_2$O$_2$ (a), 1 µM EAD2-hemin mixture with 2mM luminol and H$_2$O$_2$ (b), 1 µM hemin mixture with 2mM luminol, H$_2$O$_2$ and 0.5mM *p*-coumaric acid mixture (c), 1 µM EAD2-hemin mixture with 1mM luminol, H$_2$O$_2$ and 0.5mM p-coumaric acid(d). / CL accumulation time: 107sec.

4. Real sample tests

In prior to test the applicability of SHDs in real samples, we examined the catalytic activity of EAD2(G-quadruplex)-hemin complex in real samples (Figure S4). The CL intensity generated from EAD2-hemin complex was greatly decreased in the presence of undiluted real samples. To avoid these depression of CL signal, samples were diluted 100 fold and sensitivity test were demonstrated.

Figure S4. An accumulated CL image of Luminol-H_2O_2 reaction of EAD2-hemin complex and pure whole milk (or diluted milk) mixture. CL intensity was suppressed by whole milk. [EAD2]= 200 nM, [hemin]= 1 μM, [luminol/H_2O_2/*p*-coumaric acid]= 2mM/2mM/0.5mM/ CL accumulation time: 180sec.
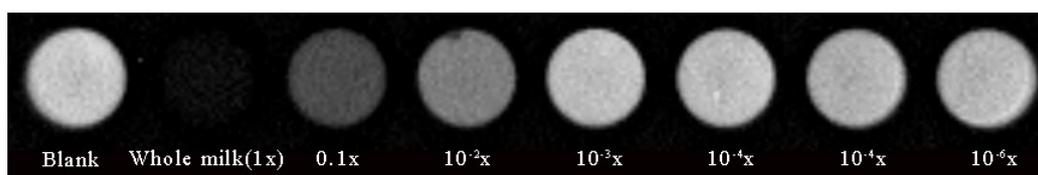

Figure S5. An accumulated CL image of Luminol-H_2O_2 reaction of SHD$_3$-hemin complex with *S*. Typhimurium spiked chicken eggs. (100-fold diluted eggs) Concentration of S [EAD2]= 200 nM, [hemin]= 1 μM, [luminol/H_2O_2/*p*-coumaric acid]= 2mM/2mM/0.5mM/ CL accumulation time: 180sec.

5. Gibbs free energy calculation

Table S1. [Hairpin]$_0$ = 100 nM, [Hemin]$_0$ = 300 nM

ΔG°_{blank}	[Hairpin]	[G-quad]	[Hemin]	efficiency
- 3.7 kcal/mol	100 nM	0.016 nM	300 nM	0.016 %
- 6.0 kcal/mol	99 nM	0.75 nM	299 nM	0.75 %
- 8.0 kcal/mol	83 nM	17 nM	283 nM	17 %
- 10.0 kcal/mol	17 nM	83 nM	217 nM	83 %

Table S2. [Hairpin]$_0$ = 5 µM, [Hemin]$_0$ = 15 µM

ΔG°_{blank}	[Hairpin]	[G-quad]	[Hemin]	efficiency
- 3.7 kcal/mol	4.962 µM	0.038 µM	14.962 µM	0.76 %
- 6.0 kcal/mol	3.7 µM	1.3 µM	13.7 µM	26 %
- 8.0 kcal/mol	0.6 µM	4.4 µM	10.6 µM	88 %
- 10.0 kcal/mol	0.02 µM	4.98 µM	10.02 µM	99.6 %

Table S3. [Hairpin]$_0$ = 100 µM, [Hemin]$_0$ = 300 µM

ΔG°_{blank}	[Hairpin]	[G-quad]	[Hemin]	efficiency
- 3.7 kcal/mol	87 µM	13 µM	287 µM	13 %
- 6.0 kcal/mol	16 µM	84 µM	216 µM	84 %
- 8.0 kcal/mol	1 µM	99 µM	201 µM	99 %
- 10.0 kcal/mol	0 µM	100 µM	200 µM	100 %

In order to calculate the individual SHD$_4$ concentration in hairpin state and in G-quadruplex state, we assumed that G-quadruplex formation free energy is – 9.2 kcal/mol and hairpin formation energy is -5.7 kcal/mol. The standard free energy change for the hairpin to turn into G-quadruplex is calculated to be -3.7 kcal/mol, ΔG°_{blank}. Bearing the energy in mind, we have calculated the actual hairpin and G-quadruplex concentrations for the given initial hairpin concentration of 100 nM, [hairpin]$_0$ and hemin concentration of 300 nM, [hemin]$_0$: Gas constant R= 8.31447 JK^{-1}mol^{-1}

$$\Delta G = \Delta G^\circ_{blank} + RT \ln \left(\frac{[G-quad]}{[haripin][hemin]} \right)$$

while [G-quad]$_0$ = 0, [hairpin]$_0$ = 100 nM, [hemin]$_0$ = 300 nM

At equilibrium, ΔG = 0,   [G-quad] = x, [hairpin] = [hairpin]$_0$ – x, [hemin] = [hemin]$_0$ – x

$$-\Delta G^{\circ}_{\text{blank}} = RT \ln \left(\frac{[G-quad]}{[haripin][hemin]} \right),$$

$$3.7 \text{ kcal/mol} \cdot 4186 \text{ J/kcal} = RT \ln \left(\frac{x \cdot 10^9}{(100-x)(300-x)} \right)$$

Solving the quadratic equation yields an exact solution for the G-quadruplex concentration at equilibrium, and results at diverse initial concentrations and $\Delta G^{\circ}_{\text{blank}}$ from -3.7 kcal/mol to -10 kcal/mol were summarized in Table 1, 2, and 3 in the above.