Supplementary Information (SI) for Sensors & Diagnostics.

Electronic Supplementary Material

Competitive Horseradish Peroxidase-Linked Aptamer Assay for Sensitive Detection of 17β-Estradiol with a New Aptamer

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Table S1 List of oligonucleotide sequences

Name	Sequences (5' to 3')
CN-Es2	CGA CTT AAG GTA TGT GAT CTT AGT TGT AGT TCA AGT CG
Es2-Apt	CTC TCG ACG ACT TAA GGT ATG TGA TCT TAG TTG TAG TTC AAG TCG T-BioTEG
C12	AGT CGT CGA GAG-BioTEG
C14	TAA GTC GTC GAG AG-BioTEG
C16	CTT AAG TCG TCG AGA G-BioTEG

Table S2 List of buffers used in ELAA

Buffer	Contents
Coating Buffer	10 μg/mL SA in 0.1 M Na_2CO_3 (pH 9.6)
Blocking Buffer	20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 10 mg/mL BSA
Washing Buffer	20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% Tween-20
Immobilization Buffer	20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% Tween-20
Assay Buffer	20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 150 mM NaCl

Table S3 ITC analysis of binding between aptamer CN-Es2 and E2

Stoichiometry (N)	K_d (nM)	ΔH (kJ/mol)	ΔG (kJ/mol)	-TΔS (kJ/mol)
1.83 ± 0.044	55.3 ± 32.0	-30.7 ± 1.65	-41.5	-10.7

Table S4 Comparison of some aptamer- or antibody-based assays for E2 detection with respect to affinity ligand, dynamic range, and detection limit

Method	Affinity ligand	Dynamic range (nM)	Detection limit (nM)	Ref.
Aptamer-based fluorescent biosensors	Aptamer CN-Es2	5 - 100	3.3	[22]
Microtiter plate-based assay	Aptamer 76-mer-G	0.013 - 200000	46	[25]
Dual-signal output sensor based on two different signal transduction mechanism	Aptamer 76-mer-G	0.1 - 200	0.059	[27]
"signal-off" PEC aptasensor mediated by enzymatic amplification	Aptamer 76-mer-G	1.0 - 250	0.37	[29]
Split aptamers regulated CRISPR-SERS LFA	Aptamer 35-mer-truncated-H	0.001 - 10000	0.00018	[30]
EXHCR-FL sensing method	Aptamer 76-mer-G	0.0015 – 2.94	0.0014	[31]
FRET-based turn-on fluorescent aptasensor	Aptamer 76-mer-G	0.35 - 35000	0.35	[32]
Label-free and sensitive electrochemiluminescence aptasensor	Aptamer 76-mer-G	0.01 - 10	0.0011	[33]
QD-FRET and competitive-based aptasensor	Aptamer 76-mer-G	0.82 - 20.5	0.22	[34]
Competitive ELAA in microplates	Aptamer CN-Es2	0.05 - 50000	0.05	This work
Intramolecular aptamer switches	Aptamer CN-Es2	100 - 1000	100	[35]
Dual-mode fluorescence and colorimetric detection	Aptamer CN-Es2	0 - 10000 or 0 - 20000	150 or 270	[36]
Photocatalytic aptamer chemiluminescent system	Aptamer CN-Es2	0 - 10000	86	[37]
Electrochemical integrate paper-based immunosensor	Antibody	0.0037 - 370	0.037	[38]
Colorimetric immunoassay with gold nanoparticle assembly and disassembly	Antibody	0.01 - 40	0.01	[39]
Fluorometric enzyme immunoassay	Antibody	0.12 - 25	0.12	[40]

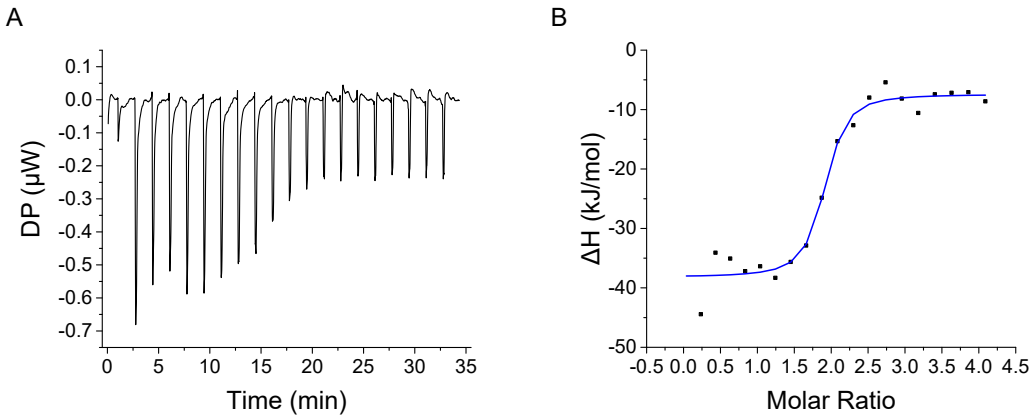

Fig. S1 ITC measurement (A) and binding isotherm fitted by one-site model (B) for the titration of E2 with original aptamer sequence (CN-Es2).

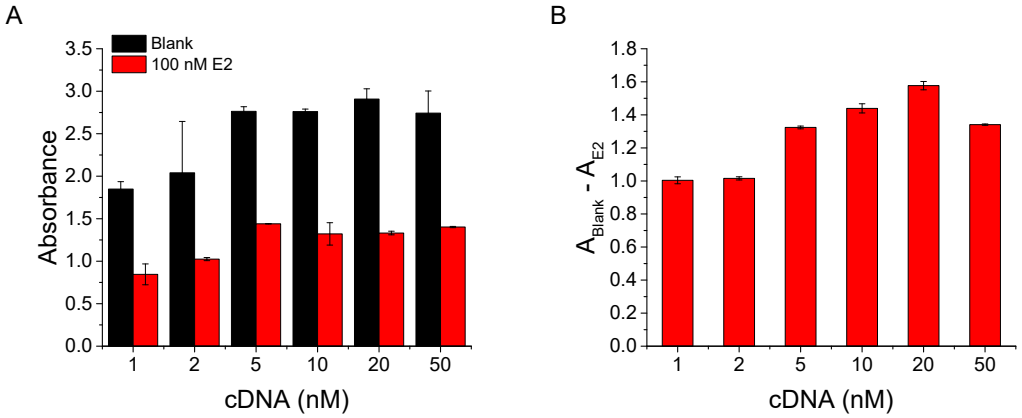

Fig. S2 Effect of the amount of cDNA on the surface of microplate. (A) The absorbance signal in the absence or presence of 100 nM E2. (B) The signal change caused by 100 nM E2 (obtained by subtracting the absorbance signal of blank sample from the absorbance signal of E2 sample). The concentration of HRP-labeled aptamer was 3 nM. Varying concentrations of cDNA C16 was used in the immobilization buffer for preparing cDNA-coated microplate. The assay buffer contained 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, and 100 mM NaCl. The immobilized cDNA and E2-aptamer complex were incubated at 4 ºC for 15 min.

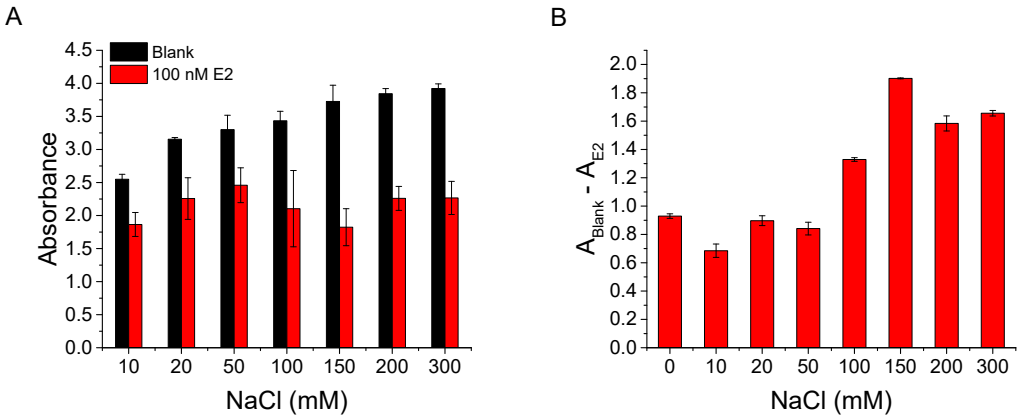

Fig. S3 (A) Effect of NaCl concentration on absorbance signals in the absence or in the presence of 100 nM E2. (B) Effect of NaCl concentration on the decrease of absorbance signal caused by 100 nM E2 (calculated by subtracting the absorbance signal of blank sample from the absorbance signal of E2 sample). 3 nM HRP-labeled aptamer was applied. 20 nM cDNA C16 was used for preparation of cDNA-coated microplate. The assay buffer contained 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, and various concentrations of NaCl. The immobilized cDNA and E2-aptamer complex were incubated at 4 ºC for 15 min.

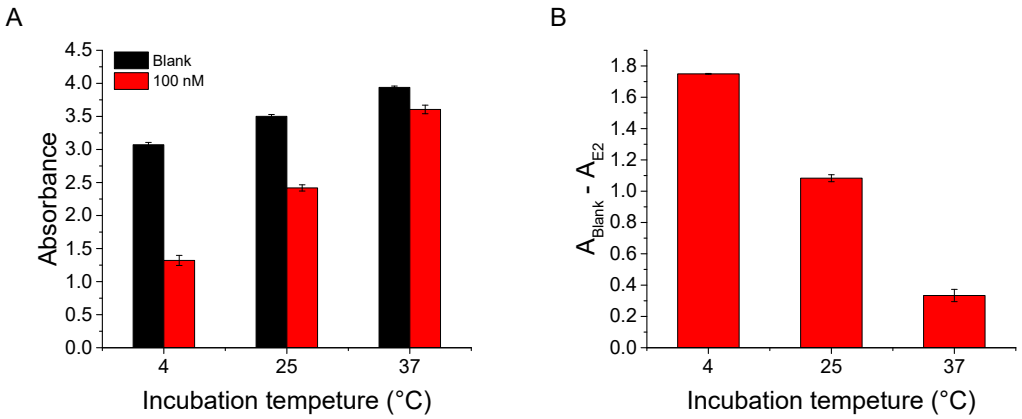

Fig. S4 (A) Effect of incubation temperature at competition process on absorbance signals in the absence or in the presence of 100 nM E2. (B) Effect of incubation temperature at competition process on the decrease of absorbance signal caused by 100 nM E2 (calculated by subtracting the absorbance signal of blank sample from the absorbance signal of E2 sample). 3 nM HRP-labeled aptamer was applied. 20 nM cDNA C16 was used in immobilization buffer for preparation of cDNA-coated microplate. The assay buffer contained 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, and 150 mM NaCl. The immobilized cDNA and E2-aptamer complex were incubated at different temperature for 15 min.

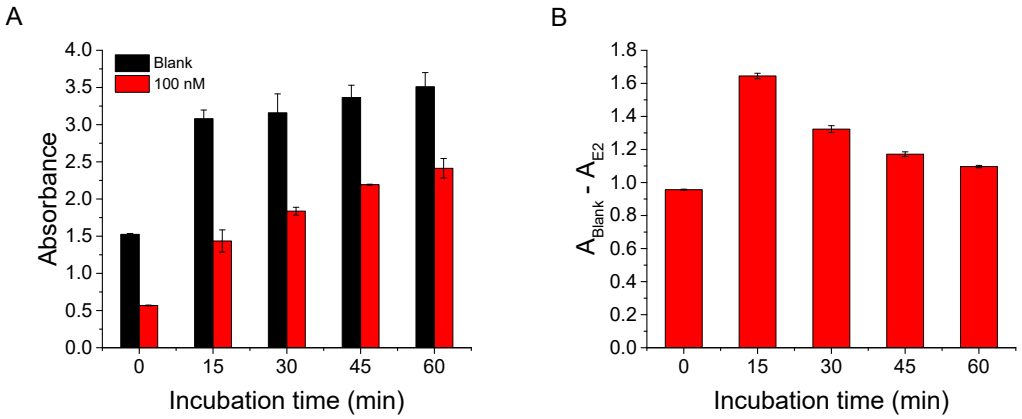

Fig. S5 (A) Effect of incubation time at competition process on absorbance signals in the absence or in the presence of 100 nM E2. (B) Effect of incubation time at competition process on the decrease of absorbance signal caused by 100 nM E2 (calculated by subtracting the absorbance signal of blank sample from the absorbance signal of E2 sample). 3 nM HRP-labeled aptamer was applied. 20 nM cDNA C16 was used in immobilization buffer for preparation of cDNA-coated microplate. The assay buffer contained 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, and 150 mM NaCl. The immobilized cDNA and E2-aptamer complex were incubated at 4 ºC.

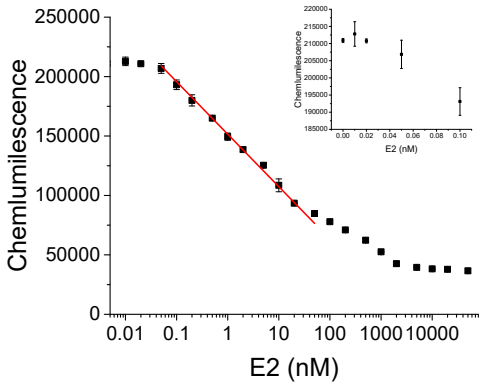

Fig. S6 Detection of E2 by ELAA with chemiluminescence-analysis mode at optimal conditions. The inset shows the chemiluminescence signals corresponding to blank sample and low concentrations of E2. The assay buffer contained 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 150 mM NaCl.

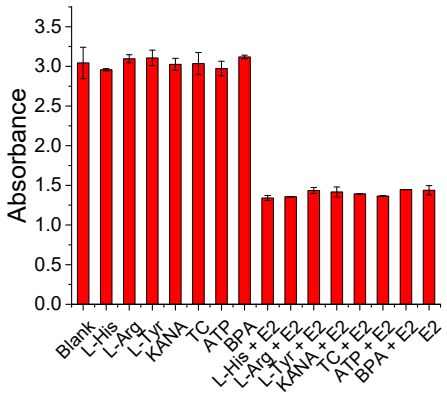

Fig. S7 Specificity test of the assay for E2 using absorbance analysis. Blank represents the system that did not contain any analytes. The concentrations of all the analytes were 100 nM.

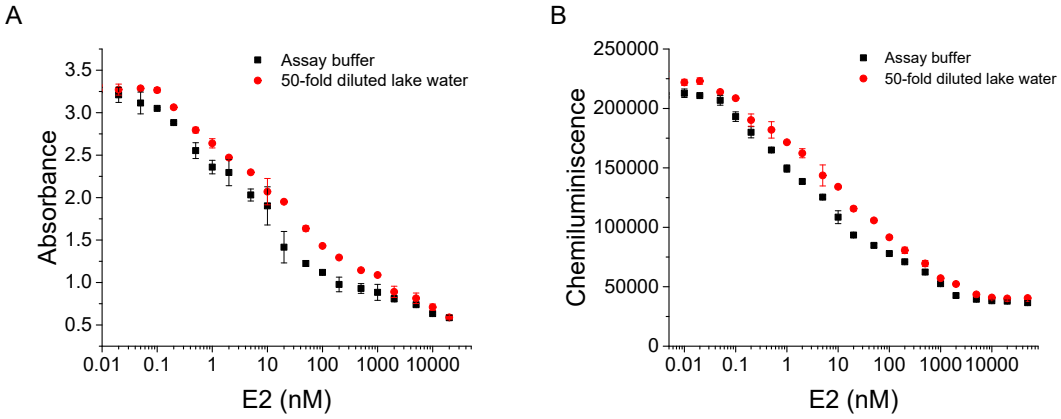

Fig. S8 Detection of various concentrations of E2 spiked in 50-fold diluted lake water. (A) the assay using absorbance analysis; (B) the assay utilizing chemiluminescence analysis.