Supporting information

Sensitive and selective detection of p53 gene based on a triple-helix magnetic probe coupled to fluorescent liposome hybridization assembly via rolling circle amplification

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Experimental section

Chemicals

The involved oligonucleotide sequences were listed below: the target DNA (**p53** DNA sequence): 5'-**TCA TAC CAC TGG AAG ACT C**-3'; the single-base mismatched DNA ($\mathbf{T_1}$): 5'-**TCA TAC CAC TGG AAG AAT C**-3'; the double-base mismatched DNA ($\mathbf{T_2}$): 5'-**TCA TAC CAC TGG AAG GAT C**-3'; the non-complementary DNA ($\mathbf{T_n}$): 5'-**GGT CTC TTG ATA GCA CTC A**-3'; the biotin modified assistant DNA probe with the sequence of 5'-**Bio-TTT TTT CGG AGA GAG AGA TAC GCC TCCG**-3' (denoted **bio-aDNA**); The hairpin DNA probe with the sequence of 5'-***CTCTCT* CGG AGT CTT CCA GTG TGA TGA *TCTCTC***-3' (denoted $\mathbf{HP_6}$); The hairpin DNA probe with the sequence of 5'-***CTCTCTC* GGA GTC TTC CA G TGT GAT GAC *CTCTCTC***-3' (denoted $\mathbf{HP_7}$); The hairpin DNA probe with the sequence of 5'-***CTCTCTCT* TGA GTC TTC CAG TGT GAT GAT *TCTCTCTC***-3' (denoted $\mathbf{HP_8}$); The hairpin DNA probe with the sequence of 5'-***CTCTCTCTC* CGA GTC TTC CAG TGT GAT GAC *CTCTCTCTC***-3' (denoted $\mathbf{HP_9}$); The hairpin DNA probe with the sequence of 5'-***CTCTCTCTCT* TGA GTC TTC CAG TGT GAT GA *TCTCTCTCTC***-3' (denoted $\mathbf{HP_{10}}$), the italic letters represent the arm sequences; The circular DNA template, with the sequence of 5'-P-**CTC TCT CTC CGG GAG GAA GGA GGT GTC TAC CAT ATA TCA TAT TCG TGA CTG GAG TGA AGC TTT TCG GAG GCG TAT**-3' (denoted $\mathbf{P_{circle}}$); the amino-modified reporter DNA probe with the sequence

of 5'-**GGA GGA AGG**

AGG TGT CTA-$(CH_2)_6$-NH_2-3' (denoted **liposeme DNA**). The hairpin DNA probe with the sequence of 5'-**CTT ACC TTA CCT TAC CAC AAC ATT CAC CGG ACC AGG AAA CAC CGC CAG GTA CCA CCC GTT TTT TTT TTT TCG GGT GGT ACC TGG CGG TGT TTC CTG GTC CGG TGA ATG TTG TGG TAA GGT AAG GTA AG**-3' (denoted **HP**)

Apparatus

The isothermal titration calorimetry (**ITC**) experiments were performed by using a MicroCal ITC200 (Microcal, GE Healthcare) to determine the thermodynamic parameters of the studied complexes. All titration were performed at 25 °C using an external water bath. The titration protocol was: 400 μL 10 μM HP_n degassed solution (10 mM PBS buffer containing 300 mM NaCl, 20 mM $MgCl_2$, pH5.5) was titrated with 200 μM degassed aDNA guest solution or 100 μM degassed p53 gene guest solution (same buffer) in a 50 μL syringe. Each titration consisted of 20 steps, 2 μL each step. The reference power and the stirring speed were maintained at 5 μcal s^{-1} and 750 rpm throughout the experiment. The resulting corrected injection heats were plotted and fitted to different models proposed in Origin 7.0 software package provided by the ITC200 calorimeter. The experiments were performed at 25 °C and at pH 5.5. In the non-linear least squares fitting of the experimental data, the binding constant (K), the number of binding sites (N) and the standard molar enthalpy change (ΔH^o) were obtained as adjustable parameters. From the values of K and ΔH^o, the thermodynamic parameters, standard Gibbs free energy change (ΔG^o) and standard

entropy change (ΔS^o) were calculated according to the basic thermodynamic equations:

$\Delta G^o = RT \ln K = \Delta H^o - T\Delta S^o$

The procedures for preparation and characterization of* SG-*encapsulated liposome and DNA-liposome probe

According to the procedure from the literature with slight modifications[1], SG-encapsulated liposome (SGEL) was prepared. The PC, Chol and DSPE with a molar ratio of 7:1:2 were added into 3 mL chloroform in the flask, and then mixed uniformly at 35 °C. The chloroform was evaporated in a rotary evaporator under vacuum for 30 min, and then the flask was flushed with argon stream for 2 h to remove any residual organic solvent traces. The dried lipid film was then rehydrated in 4 mL of SG (5×) solution and vortexed for 30 min in a bath sonicator (35 °C). To obtain narrowly distributed small unilamellar liposome vesicles, the resulting solution was extruded 11 times through a polycarbonate membrane with 100 nm pores (Nucleopore). Finally, the liposome fraction was dialyzed to remove the unencapsulated reagents using a cutoff membrane against 0.1 M PBS (pH 7.4) for 24 h at 4 °C.

The encapsulation efficiency of SG in the liposomes was estimated using the ratio of the amount of encapsulated SG in the liposomes to the amount of SG added in the preparation of the liposomes. The added SG had a volume of 1 mL and a concentration of 5×. The amount of encapsulated SG in the prepared liposomes was calculated using fluorescence to measure the concentration of SG, which was released from the prepared liposomes with 1% Triton X-100, according to the calibration curve of a standard SG solution with large amount of **HP** probes (**Figure S2**). After

calculation, the amount of encapsulated SG was 2.2×. Therefore, the encapsulation efficiency was approximately 44%. For simplicity, we designated the concentration of the SGEL as 1×. The morphology of SGEL was measured by TEM. The average hydrodynamic diameters and zeta potentials of the prepared SGEL were measured by dynamic light scattering measurements using a malvern mastersizer.

Additionally, the amine group-modified reporter DNA probe (denoted tagger DNA) was mixed with 20 μL of SG-encapsulated liposome (SGEL), 20 μL of EDC (5 M), and 10 μL of NHS (2.5 M). The mixture then was shaken fully for 1 h, and the excess EDC or NHS solution then was removed by dialysis overnight in PBS buffer at 4 °C using a cutoff membrane. Finally, the resulting liposome-DNA probe was rinsed three times with PBS to remove those components that are non-hybridized. The collected liposome-DNA probes were stored at 4 °C until use.

p53 detection in diluted cellular homogenate

The cellular homogenate was prepared according to the previous research.38. Briefly, the normal liver cells (1.0×10^6 cells) were centrifuged for 5 min at 25 °C (1000 rpm) to remove the supernatant, and the cells precipitate was resuspended in 1.0 mL of 1×Buffer C. Then, the above solution was subjected to a sonication treatment for 30 min in an ice−water bath using a probetype sonicator (200 W), and the cellular homogenate was used immediately or stored at 4 °C. Finally, p53 gene sequences were added in diluted cellular homogenate samples (1.0×10^5 cells/mL) separately. The detection procedure was the same as that described in the above mentioned experiment for p53 gene detection in pure reaction buffer.

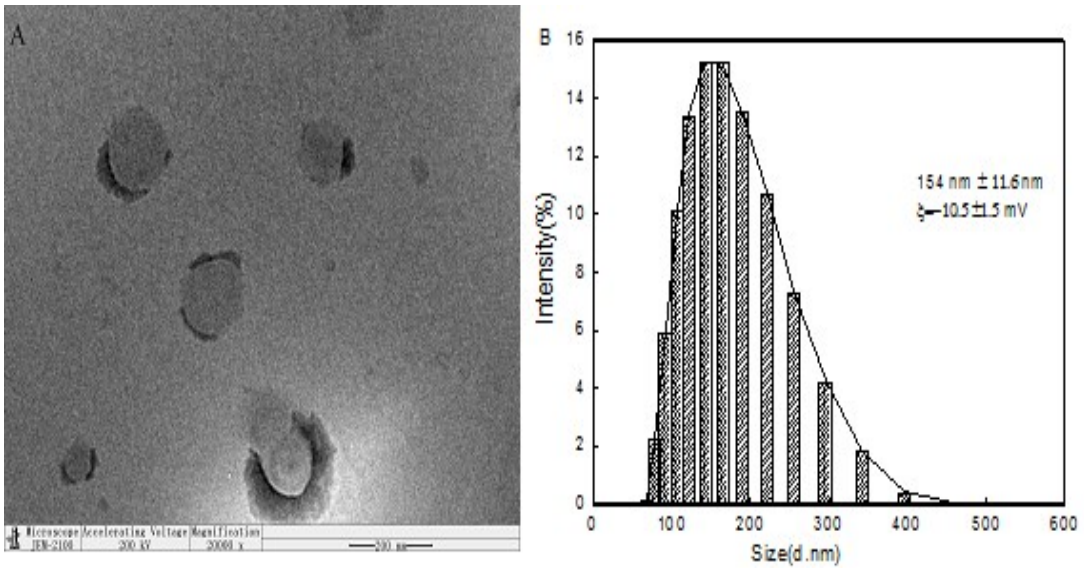

Figure. S1. (A) TEM images of the SG encapsulated into liposome; (B) dynamic light scattering (DLS) measurements of the SG encapsulated into liposome.

Characterization of the encapsulation efficiency of SG in the liposomes.

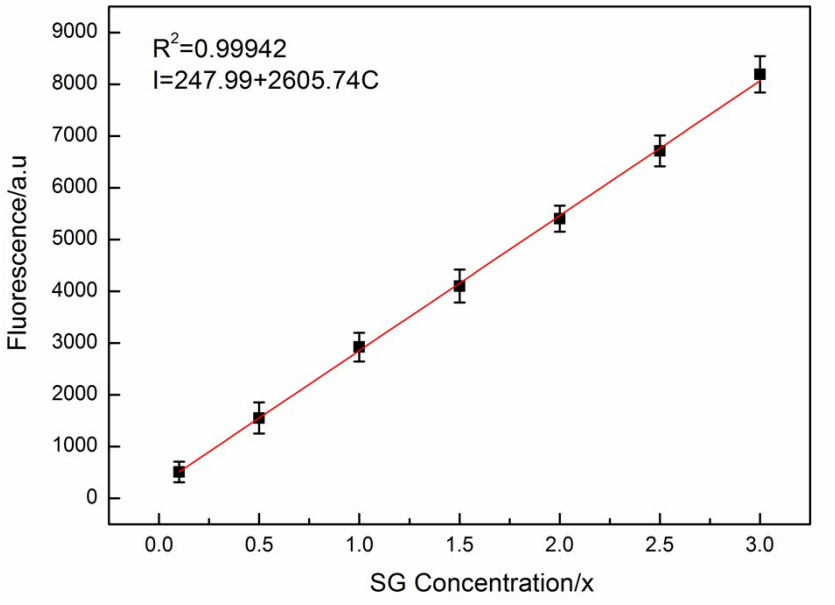

Figure S2. Fluorescence intensity versus SG concentration. Each experiment was repeated for three times, which was a representative experiment.

Characterization of the THMP probe formation (PAGE analysis)

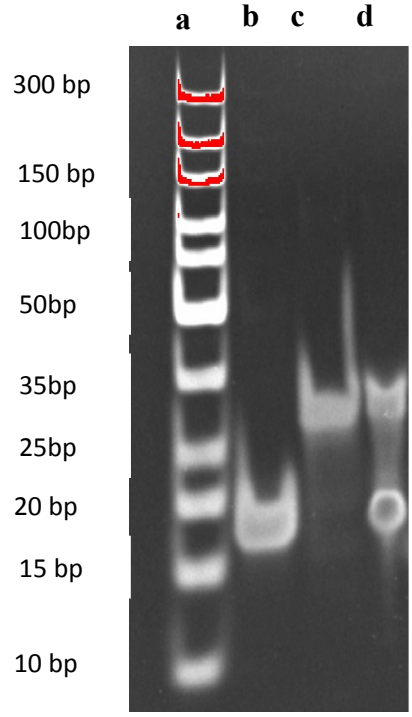

Figure S3. PAGE analysis of 10 μM Marker (a), aDNA (lane b), HP_8 DNA (lane c), aDNA + HP_8 DNA (lane d)

The isothermal titration calorimetry analysis for HP6 with p53/ aDNA.

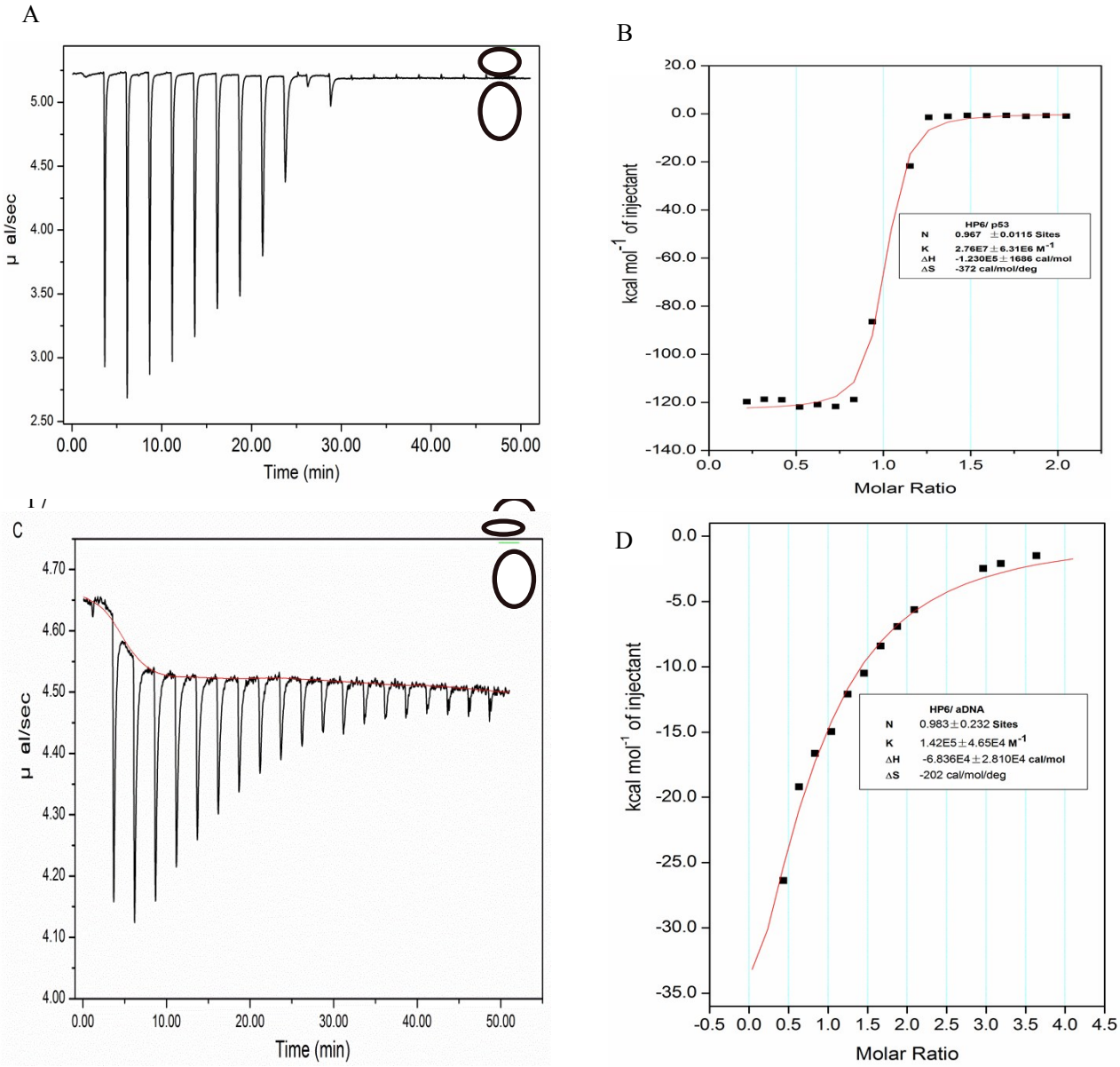

Figure S4. Typical ITC data corresponding to the inclusion interaction of 10 μM HP_6 with100 μM p53 gene (A and B) and 200 μM aDNA (C and D) at 25 °C; (A and C) Variation in the heat flow as a function of time; (B and D) integrated heat data and corresponding thermodynamic parameters.

Optimization of the reaction conditions

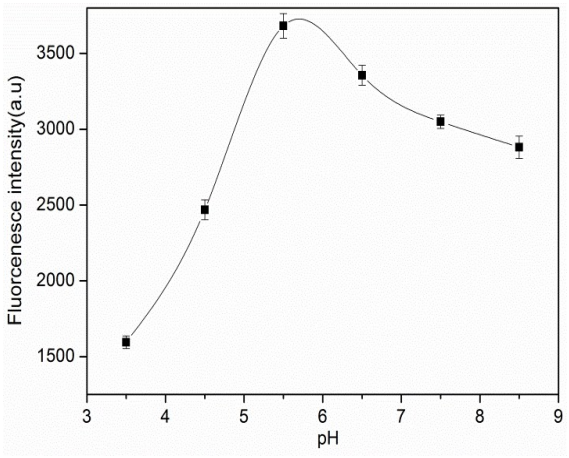

Figure S5. Effect of the buffer pH on the relative fluorescence intensities of the sensing system. Each experiment was repeated for three times, which was a representative experiment.

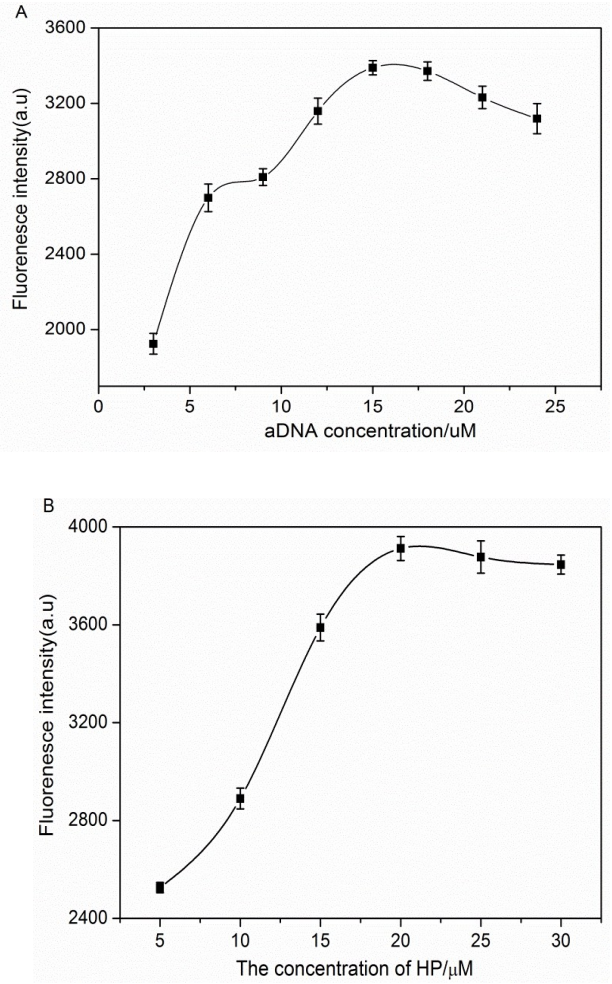

Figure S6. Effect of the concentrations of aDNA (A) abd HP_8 DNA (B) on the fluorescence intensity of the sensing system. Each experiment was repeated for three times, which was a representative experiment.

Optimization of the reaction conditions

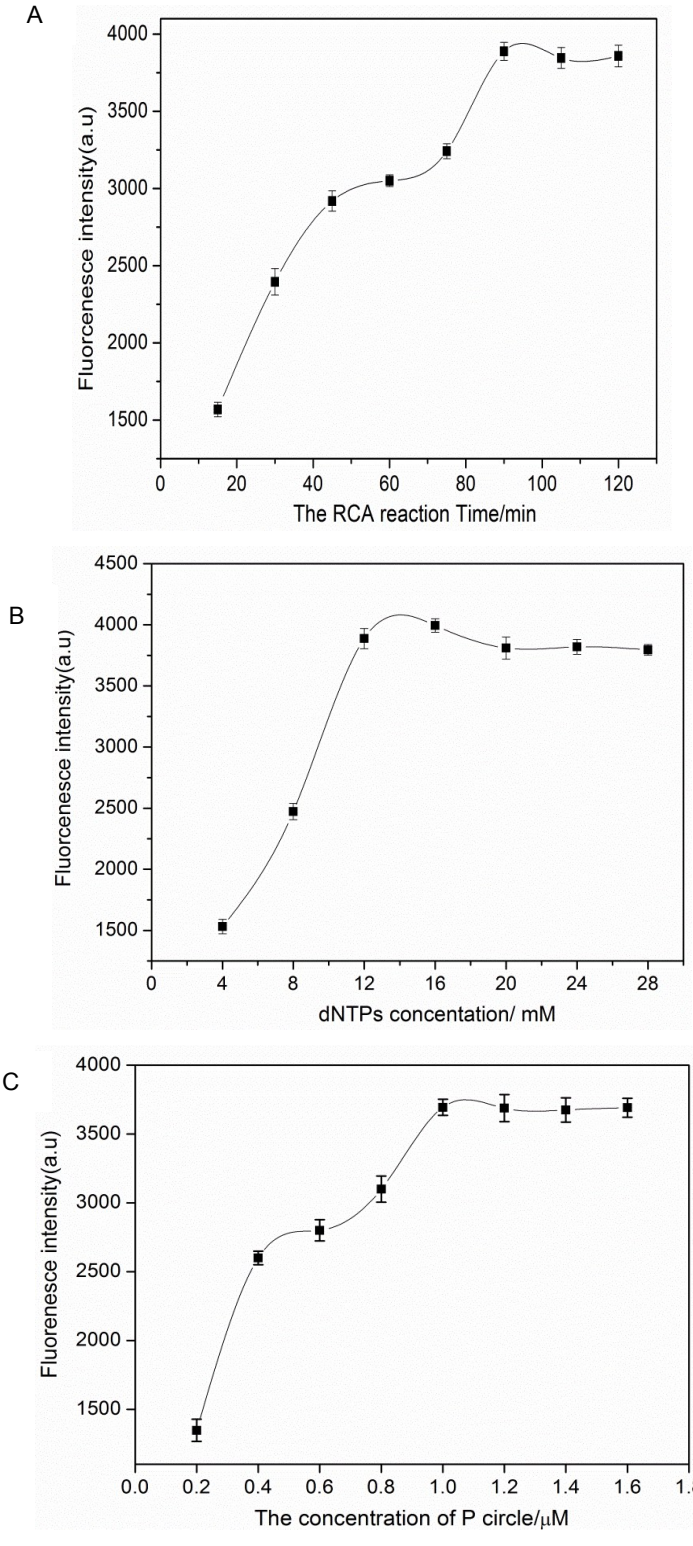

Figure S7. Effect of RCA reaction time (A), the concentration of dNTPs (B) and P-circle (C) on the fluorescence intensities of the sensing system. Each experiment was repeated for three times, which was a representative experiment.

Table S1 Association constants (Ka) and thermodynamic parameters corresponding to inclusion complex formation between HP_n probes and p53 gene or bio-aDNA

HP_n probe	p53 gene			bio-aDNA		
	Ka (mol^{-1})	ΔHº(kJ/mol)	ΔSº(J/(K*mol)	Ka (mol^{-1})	ΔHº(kJ/mol)	ΔSº(J/(K*mol)
HP_6	2.76* E7	-123.0	-372	1.42*E5	-68.36	-202
HP_7	4.38* E7	-76.88	-219	7.88*E5	-98.41	-298
HP_8	1.68* E8	-105	-312	2.24*E6	-76.84	-224
HP_9	2.77* E8	-130.9	-393	7.69*E6	-44.41	-115
HP_{10}	3.25* E8	-98.10	-285	7.07*E7	-46.85	-119

1 **Table S2.** Comparison of different methods for p53 gene detection

signal readout	amplification	LOD	linear range	selectivity	Ref.
Quartz crystal microbalance	−	0.3 nM	not given	good	2
Quartz crystal microbalance	−	0.1 nM,	0.5-25 nM	good	3
Electrochemistry	−	230 pM	1.0-95 nM,	good	4
Colorimetric	+	1 pM	1-100 pM	good	5
Electrochemistry	++	0.3 fM	1 fM -1000 pM	good	6
Electrochemistry	++	0.03 fM	0.1 fM -0.1 nM	good	7
Electrochemistry	+	0.5 fM	1 fM -1 nM	good	8
Electrochemistry	++	0.45 fM	1 fM -100 pM	good	9
Electrochemiluminescence	++	0.02 fM.	0.05-100 fM	good	10
SERS	++	21 aM,	not given	good	11
Fluorescence	+	2.5 pM	10 pM -100 nM	good	12
Fluorescence	+	80 pM	not given	good	13
Fluorescence	-	0.26 pM	0.001 -100 nM	good	14
Fluorescence	++	0.07 fM	0.1 fM - 500 fM	good	Our work

2 The "+" in the table represents with the single amplification process, the "++" in the table represents with the
3 double amplification process and the "-" in the table represents without the amplification process.

Table S3. Detection of p53 gene spiked in cellular homogenate samples by the proposed biosensor[a]

Cellular homogenate	Target DNA spiked (fM)	Target DNA detected (fM)	Recovery (%)	RSD[a] (%)
1	1	1.02	102	6.7
2	10	9.485	94.85	5.9
3	100	96.21	96.21	7.2

[a]n=3

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