

## **Electronic Supplementary Information (ESI)**

### **Detection of DNA and indirect detection of tumor cells based on circular strand-replacement DNA polymerization on electrode**

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**This Electronic Supplementary Information includes:**

- 1. Experimental Section;**
- 2. Additional results and Discussions;**
- 3. Additional figures mentioned in the manuscript.**

## 1. Experimental

### 1.1 Biology materials, reagents and apparatus

DNA sequences were synthesized by SBS-Biotech Co. Ltd. Their sequences were:

Amino-modified aptamer for Ramos cell (apt):

5'-NH<sub>2</sub>-AAC ACC GGG AGG ATA **GTT CGG TGG CTG TTC** AGG GTC TCC  
TCC CGG TG-3'

Messenger sequence (Ms, partially complementary to Ramos cell aptamer):

5'-TTA **GAA CAG CCA CCG AAC** GCG TTT-3'

Mismatched sequence (mMs):

5'-TTA **GAA CAG** G**A CCG AAC** GCG TTT-3'

Mercapto-modified hairpin probe (hpp):

SH-ATC GAT TAC CGC **GTT CGG TGG CTG TTC** TAC GTA ATC GAT-3'

Mercapto-modified primer (pri):

SH-AAA AAA AAA ATC GAT TAC -3'

In the sequences above, the segments demonstrated in **bold italic letters** stand for the complementary part between the aptamer and the target, and between the target and the hairpin probe. The segments in underlined letters stand for the two complementary ones that form the stem part of the hairpin probe. The boxed base G in the mismatched sequence indicates the one that differs from the messenger sequence.

Klenow fragment (3'→5' exo-) of *E. coli* DNA polymerase I (5 U/μL, denoted as “Klenow” for short), the buffer for Klenow-catalyzed polymerization (denoted “Klenow buffer” for short) and mixture of for dNTP (10 mM for each component) were purchased from TaKaRa Bio Inc. The Klenow buffer contained 100 mM PBS (pH 7.5), 15mM MgCl<sub>2</sub> and 1 mM DTT (dithiolthreitol). Carboxyl-modified magnetic beads (Affimag PSC 3411, diameter 0.5~1 μm, 5 mg/mL) and magnetic separation

stand were obtained from BaseLine ChromTech Research Centre (Tianjing, China). Ramos cell strain was purchased from Beijing Goldamethyst Pharm & Bio-tech Co. Ltd. (Beijing, China). Imidazole, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), tris(hydroxymethyl)aminomethane hydrochloric acid salt (tris), chloroauric acid, sodium citrate, 6-mercaptohexanol (MCH) were purchase from Sigma-Aldrich.

All the electrochemical measurements were carried out on a CHI 660C workstation (CH Instruments, Texas). Screen-printed electrodes were fabricated on a S300-M printer (Ever Bright Printing Machine Co. Ltd., Dongguan, P. R. China).

## 1.2 Aptamer recognition of the Cells

**1.2.1 The construction of the magnetic beads probe attached with aptamer and messenger sequences.** 150  $\mu\text{L}$  solution of the aptamer sequence ( $10^{-7}$  M) was mixed with 200  $\mu\text{L}$  solution of imidazole-HCl solution (pH 7.0, 0.1 M) in a 1.5 mL Eppendorf tube, and was left to stand for 30 min to form the activated aptamers. In another Eppendorf tube, 50  $\mu\text{L}$  carboxyl-modified magnetic bead suspension (5mg/mL) was mixed with 1 ml EDC solution (0.8 M), and left to stand for 60 min to obtain the activated magnetic beads.

When both were activated, the aptamer and magnetic beads were mixed, and the reaction was allowed to proceed for 12 h to form the aptamer-attached magnetic beads, which were washed thrice with 200  $\mu\text{L}$  of Tris-HCl buffer each time, and then magnetically separated on an Affimag magnetic stand. The aptamer-attached magnetic beads were then re-dispersed in 500  $\mu\text{L}$  Tris-HCl buffer (pH 4.8) to form a suspension of 0.5 mg/mL aptamer-magnetic beads.

200  $\mu\text{L}$  suspension described above was mixed with 200  $\mu\text{L}$  solution of messenger sequence ( $10^{-7}$  M). After 2 h of hybridization, the magnetic beads with aptamer-target hybridization complex were magnetically separated, and washed thrice with Tris-HCl, and then re-dispersed in 500  $\mu\text{L}$  Tris-HCl buffer (pH 4.8) to form a suspension of 0.2 mg/mL magnetic beads.

**1.2.2 Ramos Cell Culture.** Ramos Cells (CRL-1596, B-cell, human Burkitt's lymphoma) were cultured in RPMI medium supplemented first with 15% and then

with 10% fetal bovine serum (FBS) and 100 IU/mL penicillin-streptomycin. The cell density was determined using a hemocytometer prior to any experiments. The cells of specific numbers were dispersed in RPMI cell media buffer were centrifuged at 920 rpm for 5 min, re-dispersed in dye-free cell media three times, and then redispersed in 5 mL of dye-free cell media. The actual cell numbers were then extrapolated based on the cell density. During all experiments, the cells were kept in an ice bath at 4 °C to prevent internalization of any of the materials.

### **1.2.3 Aptamer recognition of Ramos cells**

Procedure of the Ramos cell recognition that released the messenger sequence was shown in Scheme 1 (B) in main text. 100  $\mu$ L suspension of aptamer-target-magnetic beads complex (0.2 mg magnet beads/mL) were added to 1 mL Ramos cell suspension of certain concentration, and was incubated under 37 °C for specific time. The suspension was then magnetically separated, and 40  $\mu$ L supernatant was sampled for the following analysis.

## **1.3 Strand-replacement DNA polymerization on the electrode**

**1.3.1 Bio-bar-code DNA attached Au nanopartirles (Reporter AuNPs).** The AuNPs (10 nm in average diameter) for attaching the reporter probes and bio-bar-code strands were prepared through the chemical reduction of chloroauric acid. 1.0% sodium citrate solution and 0.01% chloroauric acid solution (0.01%) were filtered through filter membrane with average aperture of 0.2  $\mu$ m, respectively. 1.0 mL sodium citrate solution was added dropwise into 100 mL boiling chloroauric acid solution, and stirred for 10 min in persistent boiling. The prepared gold nanoparticles were stored under 4 °C before use.

Reporter AuNPs were synthesized through incubating primers (pri,  $10^{-7}$  M), bio-bar-code strands (bbc,  $10^{-6}$  M) and AuNP dispersion in the presence of TCEP (10 mM) in 1.5 mL Tris-acetate buffer (pH 5.2). After being stirred in the dark for 16 h, the DNA-AuNP conjugate was aged in 0.1 M NaCl solution containing 20 mM Tris-acetate (pH 7.0) for 24 h, and was separated through centrifugation at 15,000 rpm for 30 min. The red oily residue was washed with 100  $\mu$ L solution containing 300 mM NaCl and 25 mM Tris-acetate (pH 8.2) and then re-centrifuged. The washing and

centrifugating were repeated twice, and the residue was re-dispersed in 1 mL solution containing 100 mM NaCl and 25 mM tris-sodium acetate, and kept under 4 °C before use.

**1.3.2 Preparation of SPE.** The analysis was carried out on a PANINT-IL-chitosan doped screen-printed electrode (SPE) as described in our previous research<sup>1</sup>. Briefly, an SPE sensor consisted of a working electrode, a counter electrode and a reference electrode. The working electrode (4 mm in effective diameter, 12.56 mm<sup>2</sup> in effective working area) was printed using a commercial graphite ink (Electrodag 323SS) doped with 0.55% (w/w) polyaniline nanotubes (PANINTs) and 7.5% (w/w) ionic liquid 1-dodecyl-3-methylimidazolium hexafluorophosphate ([C<sub>12</sub>mim][PF<sub>6</sub>]), and covered with 0.4 mg/cm<sup>2</sup> chitosan and gold nanoparticles. The counter electrode was printed with undoped Electrodag 423SS ink, and the reference electrode with Ag/AgCl ink (Electrodag 80B). Finally, an insulating layer was coated to separate the working ends and the connecting ends of the printed electrodes.

The electrodes were then dried at 60 °C, and covered with chitosan on the working electrodes, and then dried under an infrared lamp thereafter. AuNPs were deposited on the working electrodes through electrochemical reduction, which was conducted in a solution containing 6.0 mM chloroauric acid and 0.1 M potassium nitrate under -0.4 V (vs Ag/AgCl) for 400 s. 40 µL solution of hairpin probe sequences (10<sup>-7</sup> M) was cast on the working electrodes, which were placed in petri dishes for 14~16 h to allow the absorption to proceed without excessive evaporation, and then washed with 200 µL TE buffer. At last they were treated with 60 µL MCH solution (1 mM) for 2 h to block any unoccupied site on the electrode surface.

### 1.3.3 Strand-replacement DNA polymerization

The screen printed electrodes with hairpin probes were then dipped into the supernatant (2 mL circa) obtained in the magnetic separation from aptamer recognition stage. The system was incubated under 37 °C for a specific time (which was investigated in this study). Then the gold nanoparticles with primer and bio-bar-code sequences were added, and the system was allowed for

incubating for further 1 h. The electrodes were then taken out and dipped into 2 mL duplication buffer containing 5 u Klenow, dNTP (12  $\mu$ M for each component) and Klenow buffer. The duplication process was allowed to proceed for a certain time at 37 °C, and then terminated through taking out the electrodes and washing them thoroughly. The whole procedure was shown in Scheme 1 (A) in main text.

**1.3.4 Attaching of bis(1,10-phenanthroline)cobalt(II) chloride [Co(phen) for short] and DPV measurements.** To testify the occurrence of the polymerization, the assay system were constructed using unbound primer sequences (i.e. no gold nanoparticles or bio-bar-code sequences were used). Co(phen) was synthesized according to the literature<sup>2</sup> and added to the system to be tested to reach a concentration of 30.0 mM. The mixture was incubated at 37 °C for 30 min, and then subjected to differential pulse voltammetry from 0.55 to 0.05 V (vs Ag/AgCl), and the anodic peak currents at 0.25 V were taken as readout. For comparison, the blank signal (i.e. the DPV response for the hairpin probes on the screen-printed electrodes), the response before polymerization (i.e. the signal after the hybridization of the target and the reporter AuNPs) and after polymerization were measured.

**1.3.5 Attaching of hexaamineruthenium(III) chloride (RuHex for short) and chronocoulometry measurements.** After polymerization, The SPEs were immersed in 50  $\mu$ M RuHex solution for 15 min and then rinsed with PBS (pH 7.2). The chronocoulometry was carried out in PBS (pH 7.2) using a potential pulse from 0.5 V to -0.2 V (vs Ag/AgCl), with a pulse interval of 0.25 s. The charge was plotted against the square root of time, and the linear part of the plot was extrapolated back to 0 s<sup>1/2</sup> to produce an intercept on Y axis, which was taken as readout. Each measurement was performed after sufficient attaching of RuHex, and was repeated for at least three times. The average of the three closest readouts was adopted as the result.

**1.4 Performance of the cell assay in human serum.** Human serum samples of healthy adults were obtained from Qingdao Central Hospital. Ramos cell suspension was spiked into the serum to reach a specific content, and the obtained

mixture was subjected to the assay described to produce a cell content readout. The ratio of the readout to the known added content was recorded as the recovery ratio for the assay in the serum.

## **2. Addition to the Results and Discussion**

### **2.1 Suitable conditions for duplication process**

The strand-replacement DNA polymerization was affected by a series of factors. Some of them, which were associated with the nature of Klenow, had been standardized in the manual of the Klenow fragment of DNA polymerase, such as the suitable temperature (37 °C) and the suitable pH (7.4, provided by the Klenow buffer). But the other conditions that were related to the specific duplication system, including the time duration of the duplication process and the amount of the Klenow used, need to be investigated.

CC responses were plotted against time duration was examined in the range from 0 to 5 h, as shown in Figure S2. Other conditions were controlled as recommended in the manual. It was observed, as expected, that the response increased along with the extending of the duration, but the rate of increasing slowed down after the duplication was allowed to proceed for two hours, at which point the response had reached about 3/4 of the one at 3 h. After 3 h the signal did not increase further. As a balance on the consideration of the response and the speed, 2 h was chosen as the duplication time.

The amount of the Klenow enzyme used was another factor. It was investigated from 0 to 10 units, and the  $\Delta C$  responses from CC curves were plotted in figure S3. The response also increased when more Klenow was added, as expected. The concentration of the dNTP and the primer sequence, which were also directly related to the rate of the duplication, were far larger than that of the Klenow, the enzyme; thus, the rate of the Klenow-catalyzed duplication, as the common enzyme-enhanced reactions, was controlled by the concentration of Klenow. As expected, the signal increased when more polymerase was used, until the enzyme amount exceeded 5 units, which was then chosen as advised.

### **2.2 Reusability of the polymerization system**

The polymerization system used in this study consisted of Klenow (5 U), buffer for the function of the Klenow, primer ( $5 \times 10^{-8}$  M) and dNTP mixture (12  $\mu$ M for each component). The Klenow and its buffer are (at least theoretically) reusable. The reporter AuNPs and the dNTP mixture were applied in high concentrations to keep sufficient reaction rate. However, this led to a situation that the reporter AuNPs and dNTP would be far from being exhausted after one test. So, it was suggested that the use of duplication system could be repeated. A same instance of duplication solution was repeated used for eight rounds duplication. The eight individual responses averaged 8.232  $\mu$ C, with a standard error of 0.0896, showing a sufficient receptivity, meaning that the duplication system was reusable.

## 2.3 Selectivity

### 2.3.1 Selectivity for the DNA detection

The selectivity for the DNA detection was also investigated. The interactions of mismatched messenger sequence (mMs) and complementary messenger sequence (Ms) with the hairpin probe were compared through the corresponding CC curves (Figure S6). Little difference in signals was observed before and after mMs sequence of relatively high concentration ( $10^{-12}$  M) being applied in a polymerization system with all other same conditions, indicating that the mismatched sequence barely generated signal on this sensor. The signal for the messenger sequence was much larger and apparently distinguishable from the control signal or the mismatched sequence signal. A good selectivity was thus obtained on this sensor.

### 2.3.2 Selectivity for the tumor cells detection

The selectivity for the tumor cell detection was investigated as shown in Figure S9. The Ramos cells (target) of 100 cells/mL and the CEM cells (curve b, control) of  $10^4$  cells/mL were compared along with the case that no cells were applied (curve a, blank) through the corresponding CC curves. Little difference was observed between the CC response of blank and CEM cells, indicating that the unspecific cells barely generated signal on this sensor. The signal for the Ramos cells was much larger and apparently distinguishable from the blank signal or the CEM cell signal. A good



selectivity was thus obtained on this sensor.

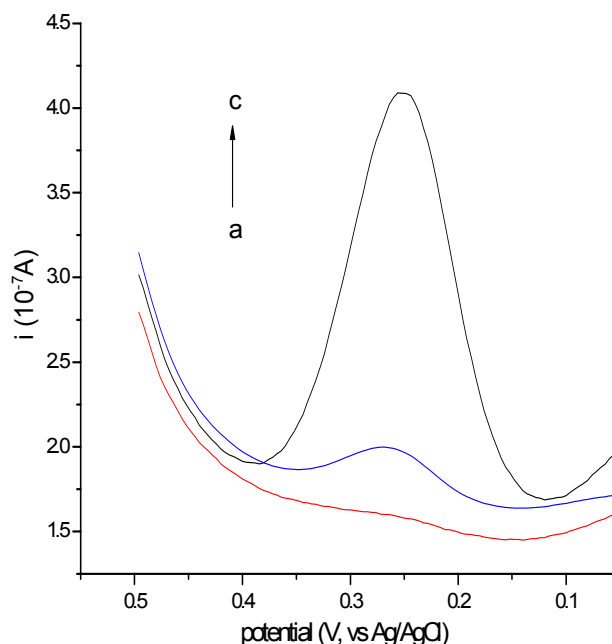


Figure S1 Comparison before and after the duplication. a) control: no target sequence, primer or Klenow was added; b) before duplication,  $10^{-10} \text{ M}$  target sequence and  $10^{-7} \text{ M}$  primer were added, but not Klenow; c) after duplication,  $10^{-10} \text{ M}$  target sequence and  $10^{-7} \text{ M}$  primer and 5 units Klenow were added, and the system was left for incubation for 3 h.

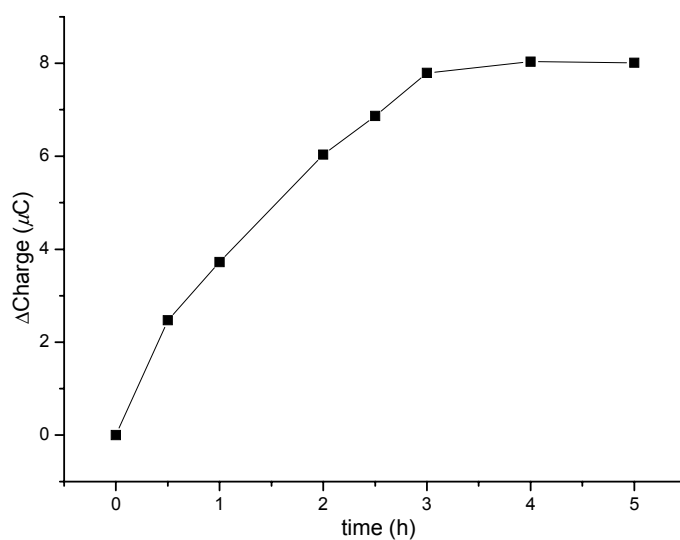


Figure S2 Relations between the duplication time and the chronocoulometric response

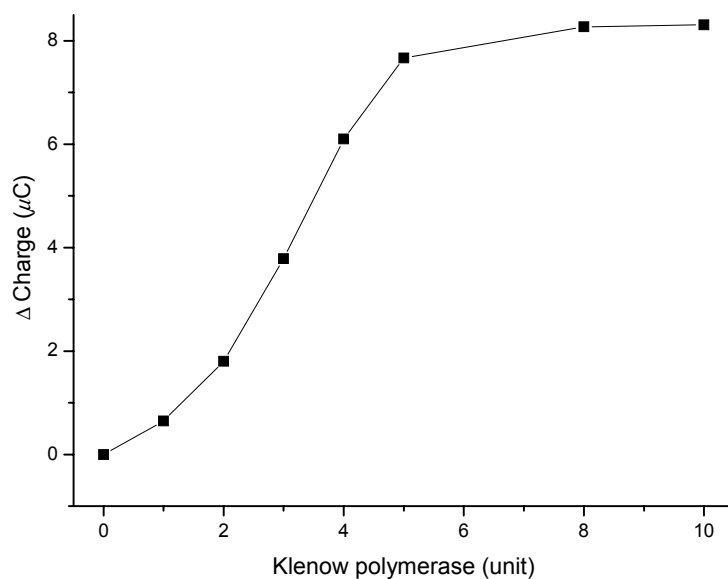


Figure S3 Relation of the CC response and the amount of Klenow polymerase

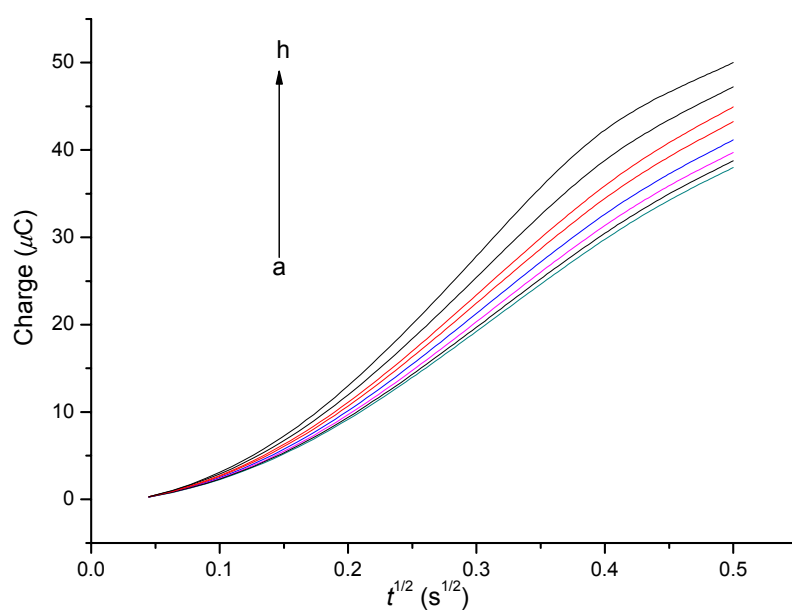
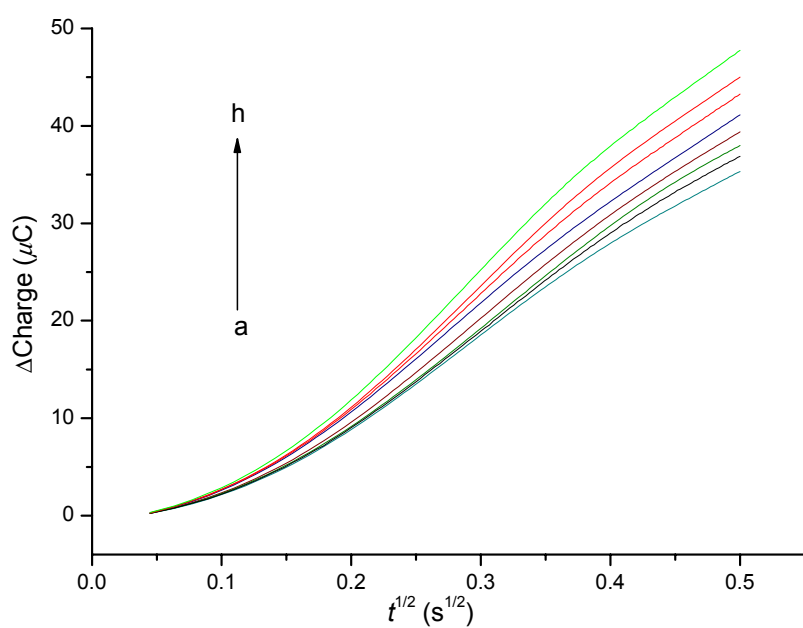
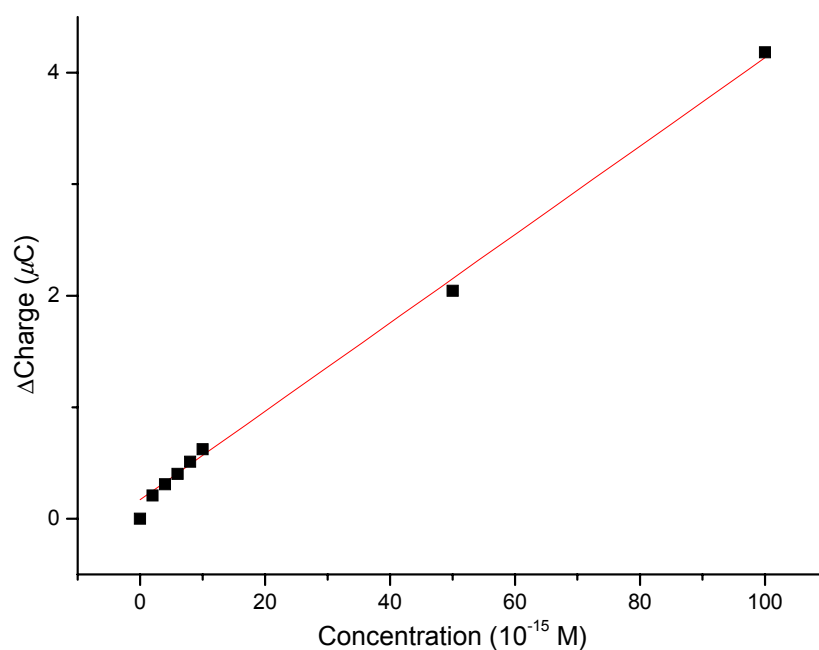


Figure S4. CC curves under different concentration of messenger DNA, after polymerization.

a: 0; b:  $2 \times 10^{-17}$  M; c:  $4 \times 10^{-17}$  M; d:  $6 \times 10^{-17}$  M; e:  $8 \times 10^{-17}$  M;  
f:  $1 \times 10^{-16}$  M; g:  $5 \times 10^{-16}$  M; h:  $1 \times 10^{-15}$



(A)



(B)

Figure S5. CC responses in the detection of DNA sequences, before duplication

(A) CC curves corresponding to different DNA concentrations

a: 0; b:  $2 \times 10^{-15}$  M; c:  $4 \times 10^{-15}$  M; d:  $6 \times 10^{-15}$  M; e:  $8 \times 10^{-15}$  M; f:  $1 \times 10^{-14}$  M;  
g:  $5 \times 10^{-14}$  M; h:  $1 \times 10^{-13}$  M

(B) Calibration curve with a regression curve of  $C = 0.0396 c_T + 0.170$

(  $C$ ,  $\mu\text{C}$ ;  $c_T$ ,  $10^{-15}$  M;  $r = 0.999$ )

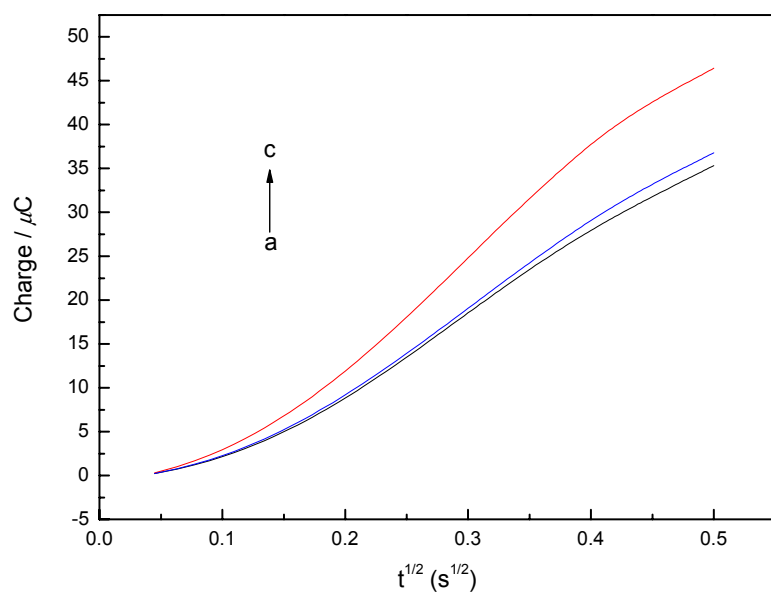


Figure S6 Selectivity in the detection of DNA sequence  
(a) No target applied; (b) mismatched messenger sequence; (c) complementary messenger sequence.

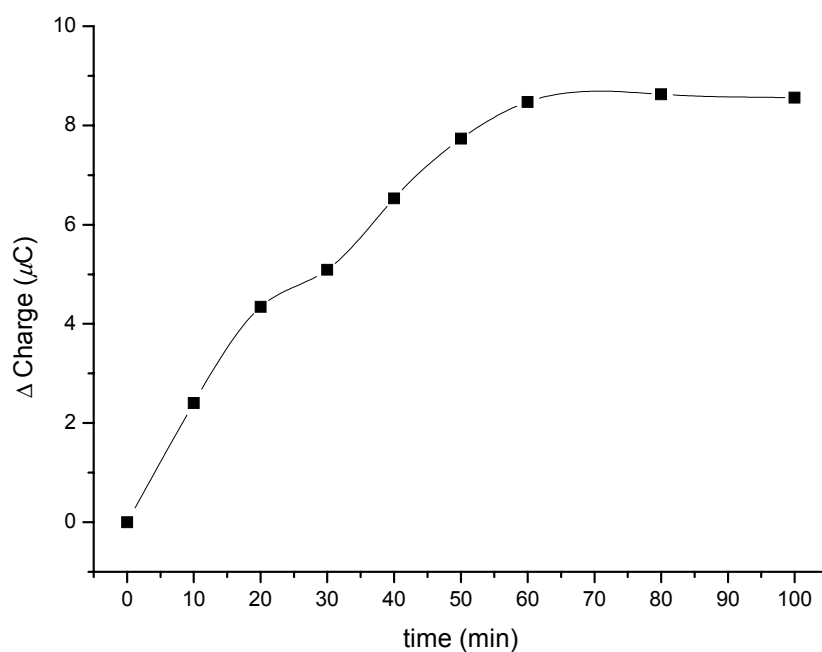


Figure S7. Relation between CC responses and the time duration for the interaction of Ramos cells and the aptamer-attached magnetic beads

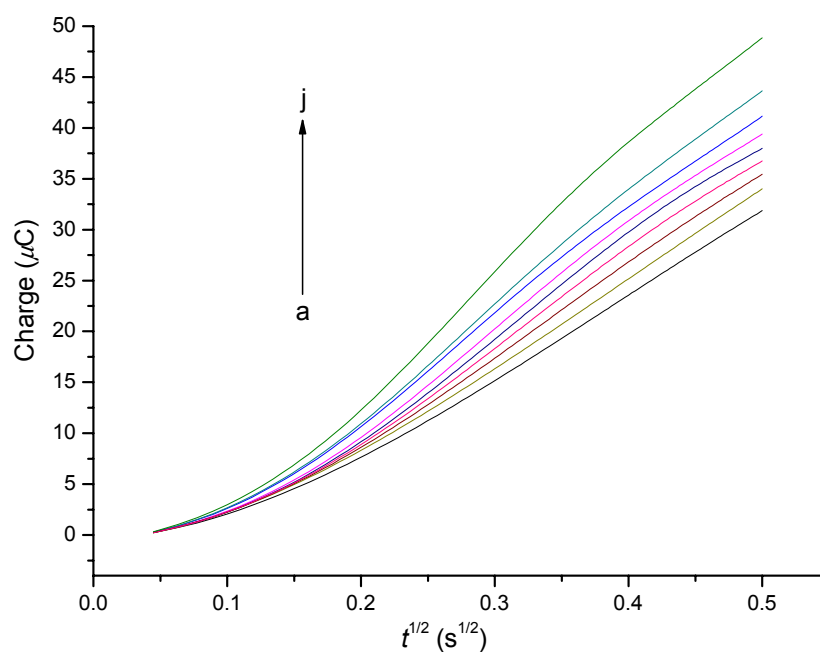


Figure S8. CC curves corresponding to different cell contents  
a: 0; b: 100 cells/mL; c: 200 cells/mL; d: 400 cells/mL; e: 600 cells/mL; f: 800 cells/mL; g: 1000 cells/mL; h: 2000 cells/mL; i: 4000 cells/mL

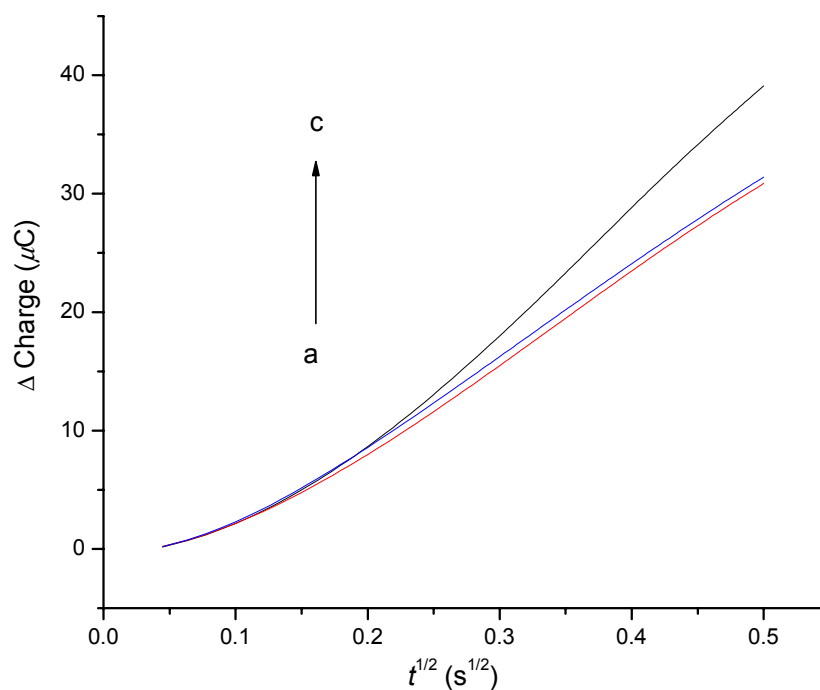


Figure S9. Selectivity in the cell detection.  
a) No cell applied; b) CEM cell; c) Ramos Cell

Table S1 Recovery ratio of the assay in the human serum samples

Nos.	Cell content detected (mL <sup>-1</sup> )	Cell content added (mL <sup>-1</sup> )	Cell content detected (mL <sup>-1</sup> )	Recovery (%)
1	0	400	342	85.5%
2	0	1,000	920	92.0%

## References

1. R. Ren, C. Leng, S. Zhang, *Bios. Bioelectron.*, 2010, **25**: 2089-2094
2. S. Niu, F. Li, S. Zhang, L. Wang, X. Li, S. Wang, *Sensors*, 2006, **6**:1234-1244.