Supporting information for

Surface Plasmon Resonance Assay Coupled with Hybridization Chain Reaction for Amplified Detection of DNA and Small Molecule

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EXPERIMENTAL SECTION

Apparatus. The surface plasmon resonance responses were measured on a SPR Navi 200 Kretschmann-type spectrometer (BioNavis Ltd., Tampere, Finland) with a light-emitting diode light source (λ=670 nm, prism refraction index n=1.61). A gold-coated glass disk mounted on a prism through a thin layer of index-matching oil form the base of a two-channel cuvette. Different samples can be added into the two independent channels. In a kinetic measurement mode, molecular adsorption on gold-coated glass disks is followed by monitoring SPR angle (θ) or angle shifts (Δθ) over time. The electrochemical surface plasmon resonance (EC-SPR) measurement was performed on DC-EC-TR-SPR instrument (Dingcheng Ltd., Changchun, China). The three-electrode system used consisted of the working electrode of interest, a Pt-wire electrode as the counter electrode, and an Ag/AgCl reference electrode. All electrochemical measurements were conducted in a standard electrochemical cell using an electrochemical analyzer (CHI660E, CH Instruments, USA). Non-denaturing polyacrylamide gel electrophoresis (PAGE) was performed on DYCZ-28C electrophoresis power supply equipped with WD-9413A gel documentation & analysis systems from Beijing Liuyi Instrument Factory (Beijing, China).

Chemicals. All oligonucleotides used in the present study were purchased either from Takara Biotechnology Co., Ltd (Dalian, China), and their sequences were provided in Supplementary Table S1. Carboxyl-modified magnetic beads (MBs) (~1.0 μm, 10 mg/mL) were obtained from BaseLine Chrom Tech Research Centre (Tianjin, China). Adenosine triphosphate (ATP), cytosine triphosphate (CTP), guanosine triphosphate (GTP), uridine triphosphate (UTP), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and EPPS were purchased from Sigma-Aldrich. Other chemicals employed were all of analytical grade and double distilled water was
used throughout the experiments. To ensure that H1 and H2 form hairpin monomers, the strands were snap-cooled before use (heat at 95 °C for 3 min and then cool to room temperature for at least 30 min).

**Table S1. DNA sequences used in the present experimental.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP aptamer (S₁)</td>
<td>5'-'NH₂-TTT TTT TT ACC TGG GGG AGT ATT GCG GAG GAA GGT-3'</td>
</tr>
<tr>
<td>Trigger DNA (S₂)</td>
<td>3'-'TCA TAA CGC CTC CTT CCA TAG GAC TCG AAT GAA CCG TCA TTC ATC-5'</td>
</tr>
<tr>
<td>Capture DNA (S₃)</td>
<td>5'-SH-(CH₂)₆-TTT TTT AGT ATT GCG GAG GAA GGT-3'</td>
</tr>
<tr>
<td>Hairpin (H1)</td>
<td>5'-Fc-ATC CTG AGC TTA CTT GGC AGT AAG TAG CGT CAC TAAG CTA CTT ACT GCC AAG T-Fc-3'</td>
</tr>
<tr>
<td>Hairpin (H2)</td>
<td>5'-Fc-CTA CTT ACT GCC AAG TAA GCT CAG GAT A CTT GGC AGT AAG TAG CT TA GTG ACG-Fc-3'</td>
</tr>
</tbody>
</table>

**Preparation of MBs modified with aptamer and trigger DNA.** The binding of the amino-modified aptamer with carboxyl-coated MBs was carried out according to the literature procedure with a slight modification. Briefly, carboxyl-modified MBs (40 mL) were transferred into a 1.5 mL Eppendorf tube and were washed three times with PBS buffer (pH 7.4). After magnetic separation, 0.1 M of EDC (150 mL) and 0.1 M of imidazol-HCl buffer were added to the MBs for 40 min to activate the carboxylate groups on the MBs. Then, the MBs were washed and resuspended in 100 µL of 10 µM amino-modified aptamer S₁, followed by incubating for 12 h at 37 °C with gentle shaking. Finally, the formed MB–aptamer conjugates were separated and washed with 200 µL of 0.01 M PBS buffer three times, resuspended in 200 µL of PBS buffer, and stored at 4 °C for further use.

200 µL suspension described above was mixed with 200 µL of trigger DNA S₂, and incubated at
37 °C for 2 h. After hybridization, the magnetic beads with aptamer-linker complex were magnetically separated, and washed with PBS buffer three times.

**ATP recognition by the aptamer and releasing of trigger DNA.** The recognition of ATP by the aptamer and trigger DNA releasing were performed as our previously reported procedures. The MB-aptamer-linker biocomplex was incubated with 100 μL of PBS containing various concentrations of ATP at room temperature for 40 min. Then the trigger DNA released from the MB-aptamer-linker biocomplex was separated into the supernatant with a magnetic field.

**Capture DNA immobilization and hybridization on Gold substrates.** Au films coated onto BK7 glass slides were purchased from Biosensing Instrument Inc. and annealed in a hydrogen flame to eliminate surface contaminants. The gold chip was dipped in piranha solution (H₂SO₄/H₂O₂, 1:1 V/V) for 5 min to make the gold hydrophilic. Addition of 30 μL of 1.0 μM capture DNA over the resultant SPR chip resulted in attachment of the capture DNA. The flow cell was washed with running buffer. After the baseline became stable, injecting of the trigger DNA released from the MB-aptamer-linker biocomplex into the fluidic channels led to a hybridized surface at 3 μL/min for 1 h. After that, the flow cell was washed with hybridization buffer for 1 h to wash away the redundant samples. Then, the hairpin sequences H₁ and H₂ were mixed and the resultant solutions were introduced into the fluidic channels at 3 μL/min for 1 h. Subsequently, the residual solutions were removed by flushing the channels with the running buffer at 10 μL/min. The surface plasmon resonance responses were measured on a SPR Navis 200 Kretschmann-type spectrometer (BioNavis Ltd., Tampere, Finland) with a light-emitting diode light source (λ=670 nm, prism refraction index n=1.61).

**Non-denaturing polyacrylamide gel electrophoresis.** The HCR reaction mixtures were loaded
onto gels contained 30% polyacrylamide (acrylamide/N,N'-methylenebisacrylamide, 29:1) in 50×TAE buffer (Tris-Acetate-EDTA, pH 8.5) followed by electrophoresis separation at 120 V for 45 min at 25°C. After staining the gel with ethidium bromide (EB), a photograph was taken with WD-9413A gel documentation & analysis systems.

Cell culture and ATP extraction. HeLa cell and K562 leukemia cell each were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 IU/mL penicillin Streptomycin. The cell density was determined by using a hemocytometer prior to any experiments. Approximately one million cells dispersed in RPMI 1640 cell media buffer were centrifuged at 3000 rpm for 5 min and rinsed with 5 mL of dye-free cell media three times and were then redispersed in cell media buffer (1 mL). Finally, the cells were disrupted by sonication for 20 min at 0 °C and the lysate was centrifuged at 18 000 rpm for 20 min at 4 °C to remove the homogenate of cell debris. For comparison, ATP levels were assayed with a modified HPLC method as described in the Supporting Information.
**PAGE and AFM characterization of the HCR.** The self-assemble in a cascading manner from H1 and H2 through HCR was investigated with native polyacrylamide gel electrophoresis (PAGE). As shown in Figure S1A, the base number of H1 (lane 4) and H2 (lane 5) oligonucleotides were almost the same, which was in agreement with our design. Significantly, the mixture of 0.1 μM H1 and 0.1 μM H2 did not cause their self-hybridization reaction (lane 6). HCR assemble was observed only in mixed H1 and H2 in the presence of trigger probe S2 (lane 9), which further verified the conditional HCR formation and assured the presence of trigger DNA to guide all HCR process, providing the basis for specific recognition. The ratio between trigger DNA and hairpin sequences was also investigated as shown in Figure S2B. When S2 was added into the H1+H2 mixture, H1 and H2 were partly consumed as a result of the HCR reaction (lane 2). The gel results also confirmed that one HCR product carried multiple monomers. Higher concentrations of S2 resulted in the increased consumption of H1 and H2 (lane 4). The average molecular weight of the resulting polymers is inversely related to the initiator concentration.

![Figure S1. An agarose gel electrophoresis image verifying the self-assembly of HCR upon the initiation of trigger DNA S2.](image)

(A) 0.1 μM S3 (lane 1), 0.1 μM S1 (lane 2), 0.1 μM S1 (lane 3), 0.1 μM H1 (lane 4), 0.1 μM H2 (lane 5), 0.1 μM H1+H2 (lane 6), 0.1 μM S2+S3 (lane 7), 0.1 μM S1+S2 (lane 8), 0.1 μM S2+H1+H2 (lane 9), 0.1 μM S1+S2+H1+H2 (lane 10); (B) different ratios
of trigger DNA and H1+H2: 1:2 (lane 1), 1:1 (lane 2) and 2:1 (lane 3), lanes 1 and 5: markers.

AFM was also used to characterize the morphology of the HCR modified on Au chips. It can be seen that a larger scale HCR product could be generated, generating a relatively rigid, and homogeneous surface morphology with the HCR product coverage on the film.

**Figure S2.** AFM height image of Au chips (a) modified with capture DNA S$_3$ (b) and the HCR products (c).

**Optimization of the concentration of the capture probe immobilized on the gold chip.** The low concentration of capture probe could not bind sufficient target, however, high concentration might increase steric hindrance for the target binding, leading to a low signal gain$^2$. Figure S3 showed the variance of SPR signal with the concentration of capture probe. In the presence of 500 fM trigger DNA S$_2$, the SPR signal increased with the increase of probe concentration from $1.0\times10^{-8}$ M to $1.0\times10^{-6}$ M, reached a maximum value and then decreased. Therefore, $1.0\times10^{-6}$ M capture DNA was employed in the following experimental.
Figure S3. Effect of capture probe concentration dependence of the SPR signal.

Optimization of the ratio of trigger H1/H2 and probe HCR reaction. The HCR reaction was initiated with introduction of a trigger strand. Amplification of the initiator recognition event continues until the supply of H1 or H2 is exhausted. We investigated the effect of the ratio of H1/H2 and trigger probe on HCR. As shown in Figure S4, the average molecule weight of the resulting polymers is increased with the increase of the ratio. Moreover, higher concentration of the H1/H2 solution results in faster speed of HCR. Taking account of the costs and reaction efficiency, the ratio of 50 for the highest concentration of trigger was chosen for our experiment.

Figure S4. Effect of ratio of H1/H2 and trigger DNA on the SPR signal. The concentration of trigger DNA is 50 fM.
Optimization of the time of HCR reaction. HCR is a time-dependent system, and the reaction time is an important influencing factor. In order to obtain higher sensitivity, the time of the HCR was investigated. As shown in Figure S5, the resonance angle changes increased as the reaction time was prolonged and reached the maximum value after 1 h. Therefore, 1 h was chosen as the optimal reaction time for HCR.

![Figure S5](image)

**Figure S5.** Effect of the HCR time on the resonance angle changes. The concentration of trigger DNA is 50 fM.

Selectivity for DNA detection. To demonstrate the specific dsDNA interaction, non-complementary DNA and one base mismatched DNA were used as control to study the nonspecific hybridization reaction. The selectivity of the present biosensor in discriminating perfect complementary DNA from single base mismatched and non-complementary DNA sequences were investigated under two concentrations as shown in Fig. S6. A significant SPR angle change was observed for the complementary sequence. The SPR angle shifts for single-base mismatched and non-complementary sequences were significantly weaker than that of the complementary sequence, producing 32% and 11% signal in comparison with the full matching target DNA in both conditions.
**Figure S6.** Specificity for the detection of DNA against blank (a), non-complementary DNA (b), one base mismatched DNA (c), and completely matched linker DNA (d).
Viability of the SPR sensor for detection of small molecules.

Figure S7. (A) Real-time resonance angle responses of HCR biosensor for ATP detection. The concentration of ATP from a to h: 0, 1.0, 5.0, 10, 20, 100, 500, 5000 nM. (B) Linear relationship between the resonance angle shifts and the ATP concentrations. The error bars are standard deviations of three repetitive measurements.

Detection of ATP in Tumor Cell Extraction with HPLC Method.

Serum samples were determined by HPLC performed on Dionex-summit system according to our previously reported method. The mobile phase consisted of 20 mM $\text{NH}_4\text{H}_2\text{PO}_4$ and 8% CH$_3$OH (V/V), degassed by vacuum extraction and ultrasonicated for 10 min. Samples (50 μL) were injected onto a C$_{18}$ bonded column (μBondapak C$_{18}$, Waters) by an automatic sample processor (ASI-100, Dionex). They were eluted at a flow rate of 1.0 mL/min, and the eluate was
monitored continuously using an UV absorbance detector at 254 nm. The absorbance peak of ATP was quantified by comparing the retention time and peak height with a known adenosine standard. The calibration curve for the determination of ATP standard was shown in Figure S6. The peak area was linear with the concentration of adenosine ranging from $1.0 \times 10^{-7}$ M to $1.0 \times 10^{-5}$ M. The regression equation was $y = 325.71x + 59.03$ (x was the concentration of ATP, μM; y was the peak height, mAU) with the correlation coefficient being 0.9963.

![Figure S8. Plot of peak height vs. the concentration of ATP.](image)

### Table S2. Comparisons of the HCR-Based method with the HPLC method for the detection of ATP in tumor cells.

<table>
<thead>
<tr>
<th>sample</th>
<th>HCR-SPR method (nmol/10^6 cell)</th>
<th>RSD (%; n=3)</th>
<th>HPLC method (nmol/10^6 cell)</th>
<th>RSD (%; n=3)</th>
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<tr>
<td>1</td>
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<td>7.56</td>
<td>4.3</td>
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<td>8.15</td>
<td>3.7</td>
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<td>4</td>
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<td>8.07</td>
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<td>5</td>
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<td>7.99</td>
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<td>6</td>
<td>7.74</td>
<td>4.2</td>
<td>7.70</td>
<td>4.3</td>
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</table>

Samples 1-3 were from HeLa cell and 4-6 for K562 cell. Each sample was analyzed in triplicate, and the results are the average values.

### References

