Supporting Information

An aptamer-based signal-on and multiplexed sensing platform for one-spot simultaneous electronic detection of proteins and small molecules

Yun Xiang,*a Xiaoqing Qian,a Bingying Jiang,b Yaqin Chai,a and Ruo Yuan*a

a Key Laboratory on Luminescence and Real-Time Analysis, Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P. R. China; Fax: +86-23-68252277; Tel: +86-23-68253172; E-mail: yunatswu@swu.edu.cn (Y.X.), yuanruo@swu.edu.cn (R.Y.).
b School of Chemistry and Chemical Engineering, Chongqing University of Technology, Chongqing 400040, P. R. China

Experimental Section

Materials: Tris-HCl, 6-mercapto-1-hexanol (MCH), HAuCl₄, lysozyme, thrombin and cytochrome c were purchased from Sigma (St. Louis, MO). Adenosine, cytidine and uridine were from Worthington Biochemical Corp. (Lakewood, NJ, USA). Disposable SPCE, comprised of a carbon working electrode (3 mm in diameter), a carbon counter electrode, and a silver pseudoreference electrode, were obtained from Zensor R&D Co., Ltd (Taichung, Taiwan). The redox-tags conjugated lysozyme (LBA) and adenosine (ABA) binding aptamers, as well as the corresponding complementary SH-cDNAs, were all ordered from Takara Biotechnology Co., Ltd. (Dalian, China) with the sequences as follows:

LBA: 5’-ATCTACGAATTCCATCAGGGCTAAAGAGTGCAGAGTTACTTAG-3’
Fc-LBA: Fc-(CH₂)₃-5’-ATCTACGAATTCCATCAGGGCTAAAGAGTGCAGAGTTACTTAG-3’
SH-cDNA₁: 5’-CCTGATGAATTCGTAGATACACTG-(CH₂)₆-SH-3’
ABA: 5’-ACCTGGGGGAGTATTGCGGAGGAAGGT-3’
MB-ABA: MB-(CH₂)₃-5’-ACCTGGGGGAGTATTGCGGAGGAAGGT-3’
SH-cDNA₂: 5’-AATACTCCCCCAGGTTTTTT-(CH₂)₆-SH-3’

All reagents were analytical grade and solutions were prepared using ultrapure water (specific resistance of 18 MΩ-cm).

**Multiplexed sensing protocol:** The SH-cDNAs were first hybridized with their corresponding aptamers to form double-stranded DNAs (SH-cDNA₁/LBA and SH-cDNA₂/ABA) separately by mixing aptamers (2.4 μM) with SH-cDNAs (2.0 μM) in the annealing buffer (10 mM Tris-HCl, 100 mM NaCl, 50 mM EDTA, pH 7.4). The mixture was heated to 95 °C for 10 min, and cooled down to 25 °C at a rate of 1 °C min⁻¹.

Gold nanoparticles (Au NPs) were electrochemically deposited on the working electrode of the SPCE according to a reported procedure.¹ ² In brief, the SPCE was first preconditioned in 20 mM Tris-HCl buffer (pH 7.4) by cycling the potential between -0.6 and 0.6 V at 0.5 V s⁻¹. This was followed by controlling the deposition potential at -0.6 V for 60 s in 10 mM HAuCl₄ containing 0.1 M KCl. The SPCE was then dried with N₂, and 30 μL of the mixture of SH-cDNA₁/LBA (1 μM) and SH-cDNA₂/ABA (1 μM) was immediately applied to the working electrode and incubated at room temperature (25 °C) for 12 h.

Next, 30 μL of 2 mM MCH was dropped on the electrode surface for 1 h. The electrode was then washed with 10 mM phosphate buffer (PB, 140 mM NaCl, 5 mM MgCl₂, pH 7.4), and the target molecules at various concentrations were added and incubated with the electrode for 1 h. After washing with PB, 30 μL of the mixture of Fc-LBA (0.5 μM) and MB-ABA (0.5 μM) were incubated with the electrode for 45 min, followed by another washing step with PB.

The electrode was then placed in a glass cell containing 2 mL Tris-HCl buffer (20 mM Tris-HCl, 140 mM NaCl, 5 mM MgCl₂, pH 7.6) and connected to an EC workstation (CHI 825C, CH Instruments Inc., Shanghai, China) for measurement. The square wave voltammograms were recorded with a step potential of 4 mV, a frequency of 25 HZ and an amplitude of 25 mV by scanning the potential from -0.50 V to +0.40 V. Data processing was made by using the “linear baseline correction” function of the CHI 852C software.
Electrochemical impedance spectroscopic characterization of the sensing platform:

![Electrochemical impedance spectra](image)

**Fig. S11** Electrochemical impedance spectra corresponding to (a) bare SPCE, (b) SPCE/Au NPs, (c) SPCE/Au NPs/SH-cDNAs-aptamers, (d) SPCE/Au NPs/SH-cDNAs-aptamers/MCH, and (e) after incubation with 100 nM lysozyme. The impedance spectra were recorded in 0.1 M KCl solution containing 5 mM (1:1) [Fe(CN)₆]³⁻/⁴⁻ with the range from 10 kHz to 50 MHz and an alternate voltage of 5 mV.

In a typical electrochemical impedance spectrum, the semicircle portion observed at higher frequencies corresponds to the electron-transfer limited process. The increase of the diameter of the semicircle reflects the increase of the interfacial charge-transfer resistance (R<sub>ct</sub>). From Fig. S11, we can see that the electrodeposition of Au NPs on the SPCE leads to a substantial decrease in R<sub>ct</sub> (from curve a to b) due to the excellent conductivity of the Au NPs. After self-assembly of the SH-cDNAs-aptamers on the Au NPs, an increase in R<sub>ct</sub> (from curve b to c) is observed. Subsequent surface blocking with MCH results in further increase in R<sub>ct</sub> (from curve c to d). The addition of the lysozyme target (100 nM) leads to the dissociation of the aptamers from the electrode surface, and the corresponding decrease in R<sub>ct</sub> is observed (from curve d to e).
Experimental optimizations for lysozyme detection:

**Fig. SI2** Influence of SH-cDNA/aptamer concentration (A), Fc-LBA concentration (B) and Fc-LBA incubation time (C) on the square wave voltammetric response of Fc in the presence of 100 nM lysozyme target (error bars: SD, n = 3). The numbers on top of the bar graphs indicate the corresponding signal to noise ratios. Measurements were carried out in 20 mM Tris-HCl buffer (140 mM NaCl, 5 mM MgCl₂, pH 7.6) by scanning the potential from -0.50 V to +0.40 V with an amplitude of 25 mV, a potential step of 4 mV and a frequency of 25 Hz.

**References:**
