Electronic Supplementary Information

Simultaneous electrochemical detection of both PSMA (+) and PSMA (-) prostate cancer cells using an RNA/peptide dual-aptamer probe

Kyoungin Min,a Kyung-Mi Song,a Minseon Cho,a Yang-Sook Chun,c Yoon-Bo Shim,b Ja Kang Ku,a and Changill Ban*a

a Department of Chemistry, Pohang University of Science and Technology, San31, Hyoja-dong, Pohang, Gyungbuk, 790-784, South Korea. Fax: 82 54 279 9929; Tel: 82 54 279 2727; E-mail: ciban@postech.ac.kr

b Department of Chemistry, Pusan National University, Jangjeon-dong, Busan, 609-735

c Department of Physiology, Seoul National University College of Medicine, 28 Yeongeon-dong, Jongno-gu, Seoul 110-799
Aptamer preparation:

**Synthesis of an A10 RNA aptamer:** The anti-PSMA aptamer (A10, GGGAGGACGAUGCGGAUCACGCAUGUUACGUCACUCCUUGUCAUCCUC AUCGGC, underlined nucleotide represents the modified pyrimidins of 2’-F UTP and 2’-F CTP) was synthesized by *in vitro* transcription \(^1\) with non-canonical ribose 2’F-UTP and 2’F-CTP using the Y639F RNA polymerase: in 100 μL of 40 mM Tris–acetate buffer (pH 8.0) containing 10 mM magnesium acetate, 0.5 mM MnCl\(_2\), 8 mM spermidine, 10 mM DTT, 1 mM EDTA, 50 μg/mL DNA template, 5 mM of each NTP and 2 μL of the purified Y639F RNAP (5 mg mL\(^{-1}\)). The reaction was carried out at 37°C for 6 hr. After adding of 10 μL of DNase I (1 mg/mL), the mixture was incubated at 37°C for 10 min to remove the DNA template. Transcribed RNA aptamer was extracted using phenol:chloroform (1:1) solution and the RNA aptamer was recovered by an ethanol precipitation method.

**Biotinylation of A10 aptamer:** The purified A10 aptamer was oxidized in the dark with 300 μL of 0.1 M NaIO\(_4\) in a 0.1 M sodium acetate buffer (pH 5.0) for 90 min at 25 °C. The reaction was stopped by the addition of glycerol (300 μL). The product was precipitated using ethanol, and finally, the oxidized RNA aptamer was coupled to 5 mM biotin hydrazide in 300 μL of 0.1M sodium acetate buffer (pH 5.0) for 4 hr at 25°C. The biotin-modified RNA aptamer was purified by a gel electrophoresis method.\(^2\) Biotinylated DUP-1 peptide aptamer (FRPNRAQDYNTN) was purchased from Anygen (Korea) and used without further purification.
A cell labeling experiment: The biotinylated A10 aptamer (0.5 μM) and DUP-1 aptamer (1.0 μM) were incubated with streptavidin coated Q655 materials (0.5 μM) for 1 hr. After the incubation process, biotin (1.5 μM) was added to each complex to block the free streptavidin on Q655. Dual-aptamer modified Q655 particles were supplemented with 0.2 mg/mL BSA to each LNCaP, PC-3, HeLa, and PNT2 cell line for 1 hr. Only PBS treated cells were prepared for negative controls. This labeling experiment was performed at room temperature with gentle shaking. After incubation of the probe-Q655 complex, each cell was washed with PBS buffer for three times. The probe-modified Q655 particles attaching to the cells were observed by optical microscope (Axiovert 40 CFL).

Figure S1. A cell labeling experiment for confirming specificity of the RNA/peptide dual-aptamer probe to prostate cancer cells.
Impedance measurement for cell detection: An electrochemical cell for EIS analysis was organized into a three-electrode configuration that consists of an Ag/AgCl reference electrode in saturated KCl solution, a platinum counter electrode, and the aptamer-immobilized Au working electrode. Faradaic impedance spectra were recorded using a PARSTAT 2263 (USA) at an open circuit potential of 10 kHz to 100 MHz. All electrochemical impedance measurements were performed in a PBS (pH 7.4) buffer with 5 mM [Fe (CN)$_6$]$^{3-/-4-}$, and impedance spectra were collected as a form of Nyquist plots. This dual-aptamer probe modified electrode was respectively immersed into various cell-suspension solutions (400 μL) for 30 min with gentle shaking (80 rpm) and checked for the specific binding of their target cell lines that was reflected in the Rct.

Figure S2. Nyquist plots for optimization of EIS detections derived from various ratios
of the A10 RNA aptamers versus DUP-1 peptide aptamers; (a) 1.5 : 1 (15 μM : 10 μM),
(b) 1 : 1 (10 μM : 10 μM), (c) 1 : 1.5 (10 μM : 15 μM), and (d) 1: 2 (10 μM : 20 μM).
Each prostate cancer cell was incubated in RPMI1640-mod media for 30 min with
gentle shaking, and BSA (0.2 mg/mL) was treated for 30 min. Detections were carried
out in a PBS (pH 7.4) with 5 mM \([\text{Fe(CN)}_6]^{3-/4-}\). Detection efficiency of the dual-
aptamer probe for prostate cancer cells can be analyzed by intervals of R_{ct} values on the
Nyquist plots. When the A10 aptamer was more supplemented than the DUP-1 to
streptavidin modified electrode for the probe (1.5:1), intervals of R_{ct} values were
gradually declined as the sequential increase of PC3 (a). However, double amount of the
DUP-1 aptamer to the A10 aptamer provides the steady increases of R_{ct} values on the
plots, and the ratio of 1:2 was then found for impartial detections for both types of
prostate cancer cells (d). More than 1:2 ratio makes difficulties of binding of LNCaP
cells to the probe (data not shown).
Figure S3. Micrographs (a) and counted cell numbers (b) of detached prostate cancer cells (PC3 and LNCaP) and a prostate normal cell (PNT-2) from the aptamer modified Au electrode with increase of cell numbers in the cell suspension.
**Table 1.** The specific detection of prostate cancer cells with co-incubation of PNT2 cells

<table>
<thead>
<tr>
<th></th>
<th>$R_{ct}$ values with $10^4$ PNT2 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^2$ cells</td>
</tr>
<tr>
<td>LNCaP</td>
<td>8443.7 ± 890.2</td>
</tr>
<tr>
<td>PC3</td>
<td>9004.4 ± 786.3</td>
</tr>
<tr>
<td>PNT2</td>
<td>5958.7 ± 660.5</td>
</tr>
</tbody>
</table>

**References**

2. P. Z. Qin and A. M. Pyle, Methods, 1999, 18, 60.