Electronic Supplementary Information

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

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Aptamer preparation:

Synthesis of an A10 RNA aptamer: The anti-PSMA aptamer (A10, GGGAGGACGAUGCGGAUCAGCCAUGUUUACGUCACUCCUUGUCAAUCCUCAUCGGC, 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). 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 Q_{655} 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 Q_{655}. Dual-aptamer modified Q_{655} 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-Q_{655} complex, each cell was washed with PBS buffer for three times. The probe-modified Q_{655} particles attaching to the cells were observed by optical microscope (Axiovert 40 CFL).

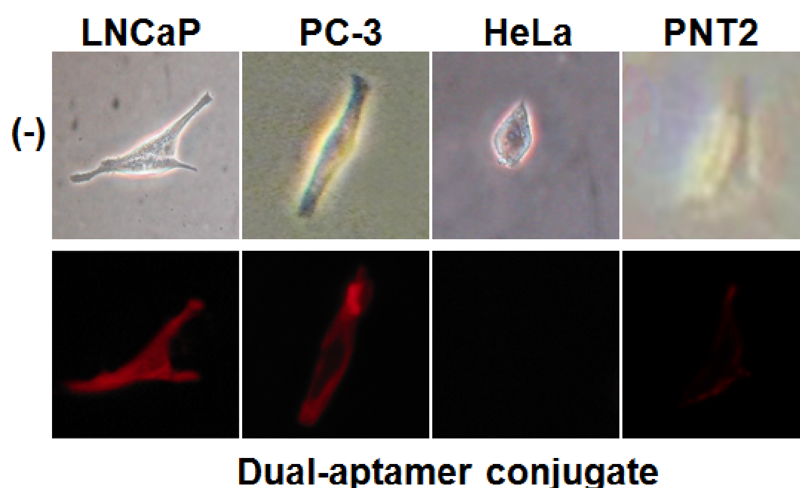


Figure S1. A cell labeling experiment for confirming specificity of the RNA/peptide dual-aptamer probe to prostate cancer cells.

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 R_{ct}.

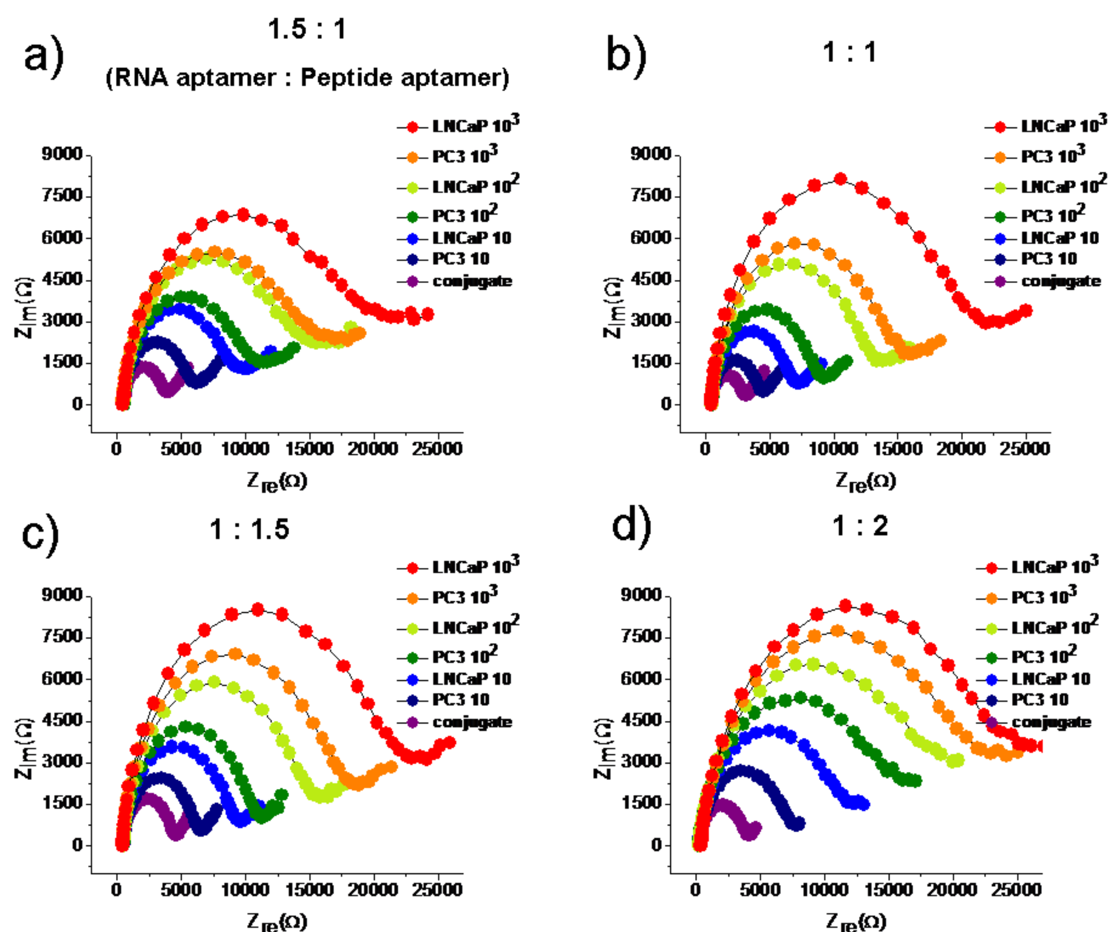
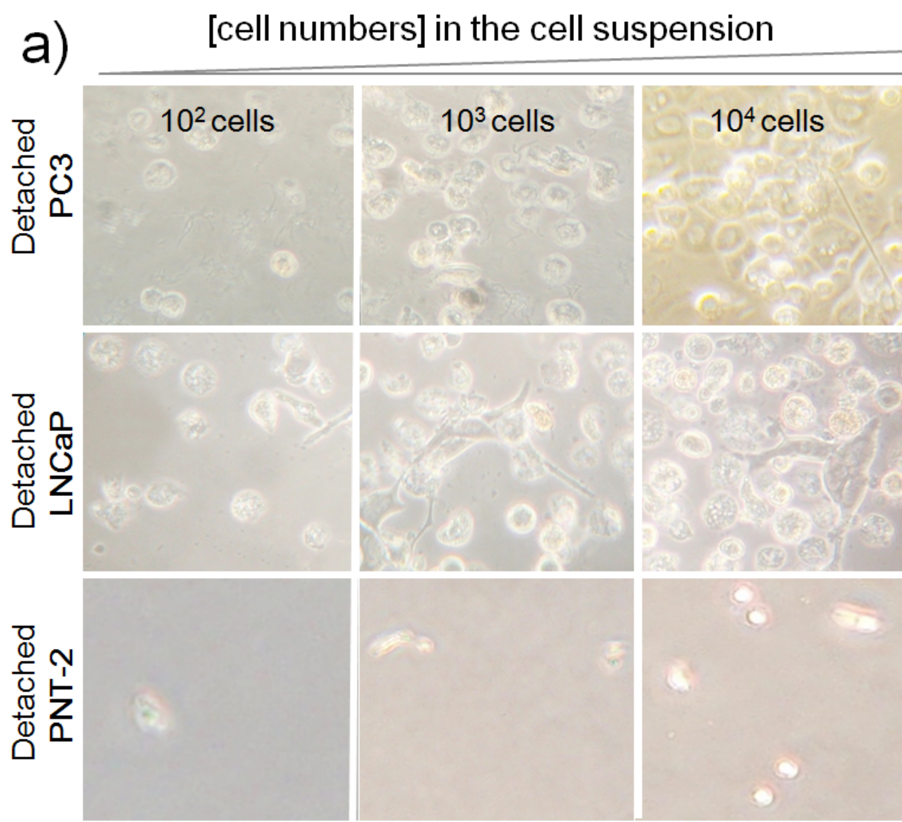


Figure S2. Nyquist plots for optimization of EIS detections derived from various ratios

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 $[Fe(CN)_6]^{3-/4-}$. 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).



b)

Cells	[cell numbers] in suspension		
	10^2	10^3	10^4
PC3	23 ± 4	112 ± 15	230 ± 42
LNCaP	19 ± 2	124 ± 23	259 ± 37
PNT-2	3 ± 0.8	7 ± 2	15 ± 3

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

	R_{ct} values with 10^4 PNT2 cells			
	10^2 cells	10^3 cells	10^4 cells	10^5 cells
LNCaP	8443.7 ± 890.2	12990.8 ± 976.9	16635.6 ± 1104.5	19237.0 ± 1302.5
PC3	9004.4 ± 786.3	12474.4 ± 954.2	15734.0 ± 1015.9	17464.9 ± 1429.8
PNT2	5958.7 ± 660.5	6375.0 ± 708.1	6977.7 ± 799.4	7875.3 ± 837.5

References

1. R. Padilla and R. Sousa, Nucleic Acids Res., 1999, **27**, 1561.
2. P. Z. Qin and A. M. Pyle, Methods, 1999, **18**, 60.