Electronic Supplementary Information

A novel immobilization strategy for electrochemical detection of cancer biomarkers: DNA-directed immobilization of aptamer sensors for sensitive detection of prostate specific antigen

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Experimental section

1.1. Materials

Gold disc working electrodes with a radius of 1.0 mm were purchased from IJ Cambria Scientific Ltd (UK). Thiolated single stranded DNA, 3-mercapto-1-propanol (MCP), were purchased from Sigma-Aldrich (UK). Oligos including probe sequence 5'- TAA TTT-3' and aptamer against PSA: 5'-ATT AAA GCT CGC CAT CAA ATA GC-3' (Mw 7004 g mol^{-1}) were custom synthesized from Sigma-Aldrich. DNA aptamer against PSA were employed for the detection of PSA elsewhere[1, 2]. Probe ssDNA (3'-TAA TTT-5') was modified with a thiol group on the 3' end to obtain HS-$(CH_2)_3$–ssDNA to assemble on gold electrodes. In addition, anti-PSA apatmer (5'-ATT AAA GCT CGC CAT CAA ATA GC-3') was modified with a thiol group to immobilized onto electrodes for DPV measurement. The HPLC DNA powders were aliquoted in 10 mM Tris-HCl, 1mM ethylenediaminetetraacetic acid (EDTA) upon receipt and stored in fridge at -20^{o}C for long-term usage. Prostate specific antigen (PSA) was purchased from Merck Millipore (UK). All the regents were used upon reception or as stated elsewhere.

The buffer for DNA probe immobilisation (IB) consisted of 0.8 M phosphate buffer (PB) + 1.0 M NaCl + 5 mM $MgCl_2$ + 1 mM ethylenediamine tetraacetic acid (EDTA) pH 7.0. The DNA hybridisation buffer and PSA incubation buffer was composed of 50 mM PB + 100 mM K_2SO_4 (pH 7.4).

1.2. Immobilization of thiolated single stranded DNA (ssDNA) on electrodes

Gold electrodes were polished with 50 nm aluminium oxide particles (Buehler, USA) on a polishing pad (Buehler) for 5 min, followed by sonication in ultrapure water, polishing on a blank polishing pad, and sonication in ultrapure water to remove any particles. Gold electrodes were rinsed with fresh piranha solution (H_2SO_4/H_2O_2, v/v 7/3) followed by rinsing thoroughly with deionized water, and readily used for electrochemically cleaning. Electrodes were electrochemically cleaned in a classical three-electrode cell as described elsewhere [3]. In brief, electrodes were immersed in H_2SO_4 (0.5 M) solution and the potential was scanned between the oxidation and reduction potentials of gold, 0 V and +1.5 V *versus* an Ag/AgCl reference electrode, with scanning rate at 0.2 V/s and step potential at 0.01 V/s for 60 cycles until there was no further change in the voltammogram.

Gold electrodes were rinsed with deionised water, dried in a stream of nitrogen and exposed to 250 μL of mixed ssDNA/MCP immobilization solution for 16 h in a humidity

chamber at 4 °C. The molar ratio between ssDNA and MCP was 1: 10 or otherwise stated, with 1 μM ssDNA in immobilization buffer. After immobilization, electrodes were rinsed in 50 mM PB + 100 mM K_2SO_4 + 10 mM EDTA (pH 7.0) to remove any remaining Mg^{2+}. In order to ensure complete thiol coverage of the gold surface and make favourable DNA conformation for hybridization, the electrodes were backfilled with MCP (1 mM, H_2O) for 1 h, followed by rinsing with ultra-pure water and slightly drying with N_2 stream.

1.3. DNA-directed immobilization aptamer sensors (DDIAS) for PSA detection

After ssDNA was immobilized on the gold electrodes, the aptamer as a complementary sequence was hybridized with the immobilized ssDNA as scaffold, so-called "DNA-directed immobilization aptamer sensor" (DDIAS). Two approaches were designed for the DDIAS for PSA detection: 'on-chip' and 'in-solution' method. In the 'on-chip' method, complementary aptamer was hybridized with ssDNA probes for 1 h at room temperature followed by incubation with PSA for 1h. The aptamer concentration was optimized at 1 μM to detect different concentration of PSA. In the 'in-solution' method: aptamer was incubated with PSA in solution for 1h, and the aptamer/PSA complexes were hybridized with ssDNA probe on the electrode. For the 'in-solution' method, a centrifugal filters (Amicon Ultra-0.5 mL 10k device, Merck Millpore, UK) was used to remove the free excessive aptamer which does not bind with PSA in solution (see Figure S1). 1. Filtration used 'in-solution' approach

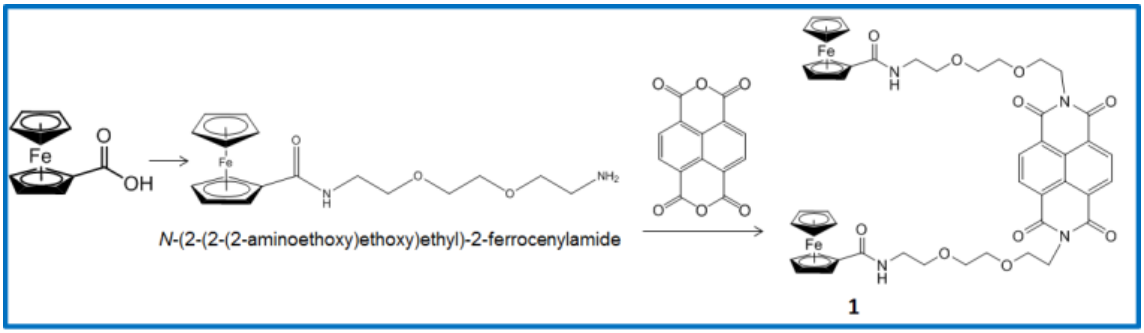
The Amicon Ultra filtration kits (Merck Millpore 0.5 mL, 10k device, UK) was used for removing the excessive aptamer for 'in-solution' approach in our DDIAS. The parameters were strictly followed with the manual of the supplier. The aptamer and PSA was incubated with the equivalent volume (250 μL each), the volume of collection sample for assay was reduced and will be top up to 500 μL in order to keep the same concentration of PSA to be analysed. The procedure for employing DDIAS for PSA detection is illustrated in Scheme 1.

In order to ensure the aptamer/PSA complexes were hybridized with the ssDNA probe, a ferrocene-functionalized double stranded DNA (dsDNA) intercalator (1 mM, THF) was introduced after binding with PSA. The ferrocene intercalator is reported to show excellent binding behaviour with dsDNA [4].

1.4. Synthesis of ferroence intercalators

The double stranded DNA intercalators *N,N*'-((((((1,3,6,8-tetraoxo-1,3,6,8-tetrahydrobenzo[*lmn*][3,8]phenanthroline-2,7-diyl)bis(ethane-2,1-diyl))bis(oxy))bis(ethane-

2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))diferrocenamide was obtained from *N*-(2-(2-(2-aminoethoxy) ethoxy)ethyl)-2-ferrocenylamide coupling with 1,4,5,8-Naphthalenetetracarboxylic dianhydride. *N*-(2-(2-(2-aminoethoxy) ethoxy)ethyl)-2-ferrocenylamide was synthesized from ferrocenecarboxylic acid and 2,2'-(ethylenedioxy)bis(ethylamine). The synthesis scheme was illustrated in Fig. S2. The products were characterized with ^{1}H and ^{13}C nuclear magnetic resonance (NMR), mass spectrometer and infrared spectrometer to confirm the structure of the compound.



Scheme S1. Illustration of synthesis of double-stranded DNA intercalator.

1.5. Electrochemical characterization

All measurements used a three-electrode cell, with an $Hg/HgSO_4$ (K_2SO_4 sat.) reference electrode against which all potentials are quoted, and a Pt counter electrode (BASi, USA). Before measurement, electrodes were placed into EIS buffer for 1 h to stabilize.

Electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV) measurements were performed using a μAutoLab III / FRA2 potentiostat (Metrohm, The Netherlands). A conventional 3-electrode configuration was used consisting of a 1.0 mm radius gold disc working electrode (CH Instruments, USA), a platinum wire counter electrode (ALS Instruments, Japan) and a Hg/Hg_2SO_4 (K_2SO_4 sat.) reference electrode (BASi, USA) placed into a salt bridge and against which all potentials are quoted.

EIS was measured in a solution of 2 mM $K_4[Fe(CN)_6]$ / 2 mM $K_3[Fe(CN)_6]$ in 50 mM phosphate buffer (PB) + 100 mM K_2SO_4 pH 7.0, ionic strength 447 mM. The reference electrode was connected via a salt bridge filled with 50 mM PB + 100 mM K_2SO_4 (pH 7.0). The impedance spectrum was measured over the frequency range 100 kHz to 100 mHz, with a 10 mV a.c. voltage superimposed on a d.c. bias of -0.195 V, corresponding to the formal potential of the redox couple.

Differential pulse voltammetry (DPV) measurements were performed by placing electrodes in 100 mM PB with Ag/AgCl as reference electrode. DPV scans run between -0.2

V and 0.7 V *vs* Ag/AgCl (scan rate 0.05 V/s, step potential 0.005 V, modulation amplitude 0.05 V, modulation time 0.05 s, interval time 0.1 s).

As shown in Fig. S1, change of charge transfer resistance was enhanced by incubation with intercalator for the detection of PSA with 'in-solution' approach, which was in agreement with our previous study [5]. The custom synthesized intercalator has potential to further improve the sensitivity of biosensors.

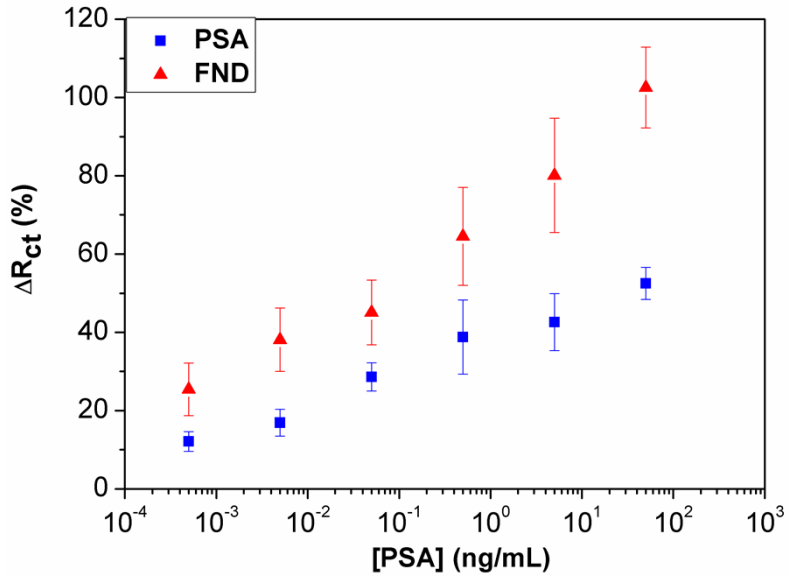


Fig. S1 Enhanced signal of the shift of charge transfer resistance of DDIAS for detection of PSA after incubation with ferrocence intercalators

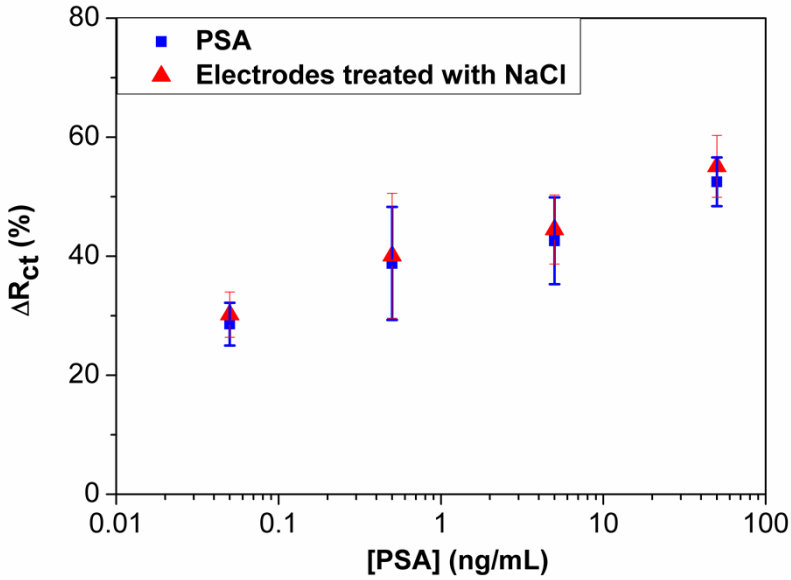


Fig. S2 Changes of charge transfer resistance for the detection of PSA within dynamic range before and after incubation with 5M NaCl solution

References

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