Supplemental information

A Hairpin DNA Aptamer Coupled with Groove Binders as a Smart Switch for a Field-Effect Transistor Biosensor

Tatsuro Goda and Yuji Miyahara*

Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University (TMDU), 2-3-10 Kanda-Surugadai, Chiyoda, Tokyo 101-0062, Japan

1. Configuration of extended gate FET device

The source and drain of a commercially available FET (2SK241, Toshiba, Japan) were connected to a real-time FET analyzer (Optogenesis, Saitama, Japan, Figure S3) at the condition of I_S = 600 μA and I_{DS} = 100 μA (i.e., V_D = 1.0 V) and the gate of the FET was extended to a gold electrode on a separate chip.

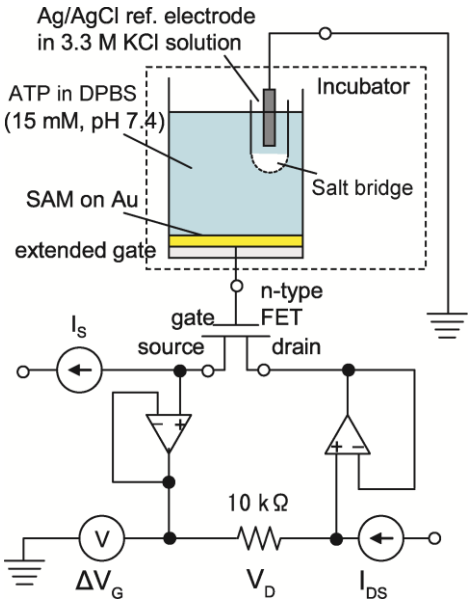

Figure S1. Circuit diagram of the extended gate-FET device.

2. Probe density of the ATP-binding aptamers

To determine the probe density of the oligo-DNA aptamers in the mixed SAM on the gold electrode, chronocoulometry (CC) was performed at a pulse period of 1000 ms and the potential stepped from 125 mV to −300 mV (vs. Ag/AgCl) in 15 mM DPBS with/without 50 μM hexammine-ruthenium (III) chloride (RuHex) using an Autolab PGSTAT 302 potentiostat (Eco Chemie, Utrecht, The Netherlands). The charge Q as a function of time t from the potential step is the sum of the reduction of RuHex

diffusing from solution, the double-layer charge and the charge due to reduction of the surface confined RuHex, so that Q is given by the integrated Cottrell equation as follows:

$$Q = 2nFAD_0^{1/2}C_0^{*}t^{1/2}/\pi^{1/2} + Q_{DL} + nFA\Gamma_0 \quad \text{(S1)}$$

where n is the number of electrons per molecule for reduction (n = 1), A is the active area of the electrode, D_0 is the diffusion coefficient, C_0^* is the bulk concentration of RuHex, Q_{DL} is the capacitive charge, and Γ_0 is the amount of surface confined redox marker. Figure S2 shows the CC response curves for the mixed SAM. Extrapolation of a least squares fit to the linear part in a plot of Q versus $t^{1/2}$ was used to determine the y-intercept. Assuming Q_{DL} to be approximately equal in measurement with/without RuHex, Γ_0 was calculated from the difference in the y-intercept values. The surface density of DNA aptamers (Γ_{DNA}) was determined from Γ_0 as follows:

$$\Gamma_{DNA} = \Gamma_0(z/m)N_A \quad \text{(S2)}$$

where z is the charge on the redox molecule (z = 3) and m is the number of bases.

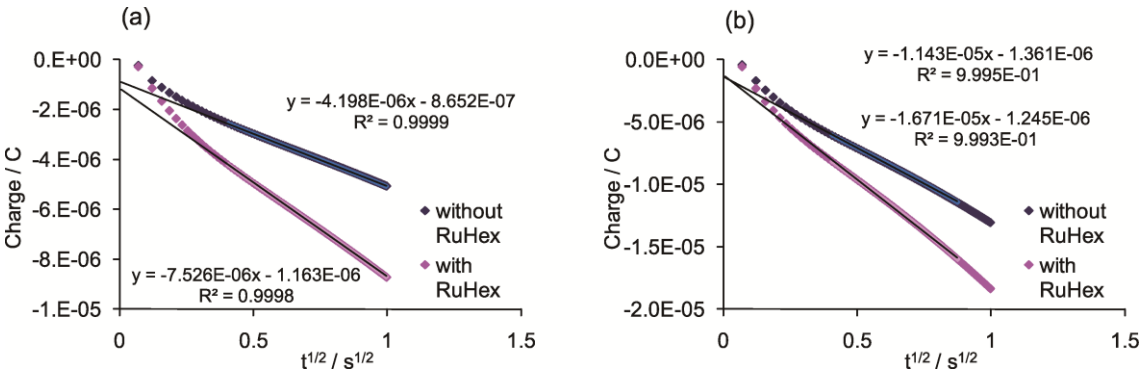

Figure S2. Chronocoulometric response curves for (a) sh-aptamer/MCH SAM- and (b) MCH SAM-modified gold electrodes in the absence and presence of 50 μM RuHex in 15 mM DPBS. The lines represent the fit to the data used to determine the y-intercept at $t^{1/2}$ = 0.

3. Packing density of the SAM

Packing density of the SAM was determined by cyclic voltammetry using the Autolab PGSTAT 302 potentiostat. A conventional three-electrode cell, consisting of a gold electrode chip as a working electrode, a platinum wire (0.25 mm diameter, 99.98%, Nilaco, Tokyo, Japan) as a counter electrode and an Ag/AgCl reference electrode (immersed in 3.3 M KCl aqueous solution with Ag^+) was used with a salt bridge. Desorption of an alkanethiol SAM was performed by scanning the potential from −0.2 to −1.2 V at a scan rate of 20 mV/s in 0.5 M KOH in 3.3 M KCl aqueous solution. All voltammograms were taken after the sample solutions were purged with nitrogen gas for 20 min. All potentials in this paper were referred to the reference electrode. The reduction peak starting from −0.8 to −1.0 V in the first scan was attributed to charge transfer from the electrode to the thiols (Figure S3). Therefore, the number of alkanethiols per unit area (γ, nm^{-2}) was calculated as:

$$\gamma = Q_{SAM} N_A / F \quad \text{(S3)}$$

where Q_{SAM} is the total surface charge density determined by the peak area, scan rate and electrode surface area; F is the Faraday constant; and N_A is Avogadro's constant.

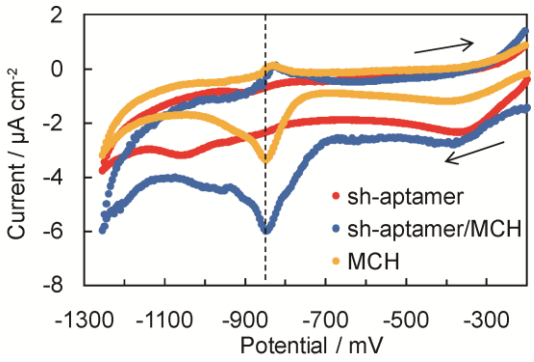

Figure S3. The first scans of cyclic voltammograms of the aptamer- or SAM-coated gold electrodes.

3. Identification of the structural transition of the sh-aptamer aptamer

The epifluorescent images were taken using a fluorescence microscope (Nikon Eclipse Ti) equipped with a 10× objective lens, a super high pressure mercury lamp (HBO 103W/2 N, OSRAM, Regensburg, Germany), and L640-Epi / LF635-A-000 filters. The electrodes were stained with 10 μM TO-PRO-3 iodide (Life Technologies) in 15 mM DPBS for 30 min. As shown in Figure S4, strong fluorescence emissions from TO-PRO-3/dsDNA complexes were solely observed on the sh-aptamer-contained SAM because of the absence of ATP-induced denaturation of the sh-aptamer. The fluorescent emission disappeared after incubation of the sh-aptamer with ATP at 37 °C. From the image analysis, the average fluorescence intensity was statistically significant on the sh-aptamer surface before and after incubation with ATP (Figure S5).

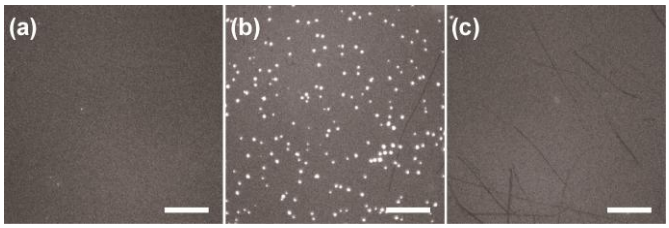

Figure S4. Identification of the closed-loop and open-loop hairpin DNA conformations on the gold electrode using epifluorescent micrographs with the indicator dye (TO-PRO-3) specific for dsDNA. (a) bare electrode; (b) the sh-aptamer/MCH-immobilized electrode before incubation with ATP; and (c) the sh-aptamer/MCH-immobilized electrode after incubation with ATP. Scale bar: 100 μm

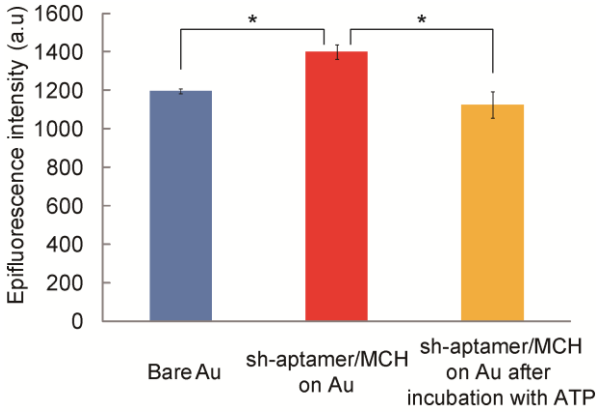

Figure S5. The average epifluorescence intensity of the gold electrodes in the presence of TO-PRO-3 indicator dye for dsDNA. * $p < 0.01$.