Electronic Supporting Information

Hg(II) Interactions with T-rich Regions in Oligonucleotides: Effects of Positional Variations on the Electrochemical Properties.

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Monolayer preparation. 25μM (30μL) solutions of ODN **I-III** were prepared in the 20 mM MOPS buffer (pH = 7.4, 150 mM $NaClO_4$). The gold electrodes were cleaned according to the reported procedure and incubated in the ODN solutions for 3 days [1]. Then, the electrodes were removed, washed thoroughly with the MOPS buffer solution and incubated in 1 mM 6-mercaptohexanol (MCH) in 20 mM MOPS buffer containing 150 mM $NaClO_4$ for 2 hrs. Subsequently, ODN/MCH modified gold electrodes were incubated in 10 μM $Hg(ClO_4)_2$ solution for 2 hrs. The electrodes were washed with 20 mM MOPS buffer after the immersion and mounted into an electrochemical cell with 3-electrodes configuration. The EIS measurements were performed on all the films.

ODN film thickness

In order to calculate the thickness of ODN film on gold electrode, high resolution XPS measurements were done. The XPS spectrum from a bare gold substrate is used as an intensity reference. The equation (S1) was used to calculate the thickness of the ODN film on the gold electrode. The intensity of the gold substrate signal, I_{Au}, is given by the intensity from a clean gold substrate, I_{Au}^0, attenuated by the ODN film of thickness t [2] as

$$I_{Au} = I_{Au}^{0} \exp\left[-\frac{t}{L_{Au}}\right] \quad \text{(S1)}$$

Where, L_{Au} designates the average practical effective attenuation length for electrons from Au in ODN film [3].

Preparation of ODN microarrays

Preparation of the ODN micro-arrays was done by using a spotting robot (SpotBot3, Telechem, Sunnyvale, CA) equipped with Megasonic Wash Station. The prepared ODN solutions were loaded into the cells of the Arrayit microplates (Sunnyvale, CA). Deionized water/ethanol mixture of 9:1 ratio was used as the wash buffer for the 946MP2 pin (Sunnyvale, CA). Humidity was maintained between 85-95% inside the chamber during the printing process. The detailed spotting parameters are as:

Pin configuration: 1×1

Spot spacing (center to center): 140 μm

Pre-print spots per sample: 10

Sample loading time: 5.0 s

Pre-print time: 0.0 s

Print time: 0.2 s

Number of wash/dry cycles: 5

Wash/dry duration: 2.0 s

Last cycle wash duration: 5.0 s

Last cycle dry duration: 10 s

After printing, the substrates with microarrays of ODN were placed on top of filter paper moistened by MOPS buffer (pH 7.4) inside a Petri dish. The Petri dish was then wrapped with parafilm and incubated for 3 days. The substrates were then removed and rinsed thoroughly using MOPS buffer and blown dried using nitrogen gas. The ODN microarrays are exposed to Hg(II) solution for 2 hours after SECM measurement and rinsed with MOPS buffer in prior to SECM measurement on the same substrate with ODN/Hg(II) modifications.

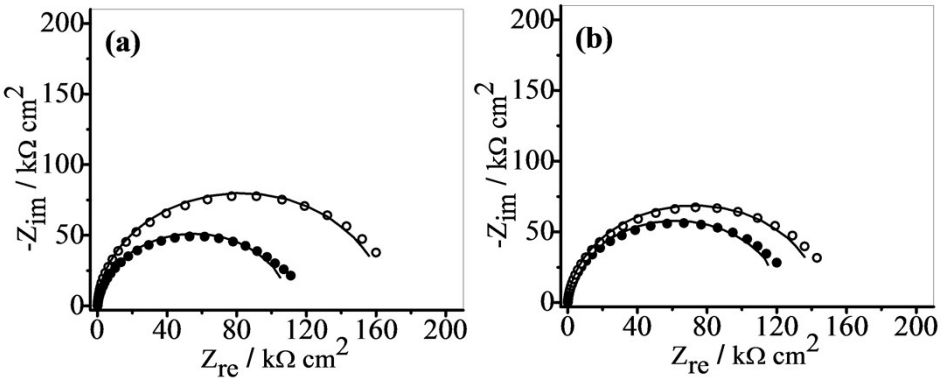

Fig.S1. Representative Nyquist plots ($-Z_{im}$ *vs.* Z_{re}) for ODN films before (●) and after (○) incubating in 20 mM MOPS buffer containing 150 mM $NaClO_4$ and 10 μM Hg(II) at pH = 7.4 for (a) ODN **II** and (b) ODN **III**. Experimental data is shown as scatters with calculated solid lines fitted using the equivalent circuit as shown in the inset of Fig.1a.

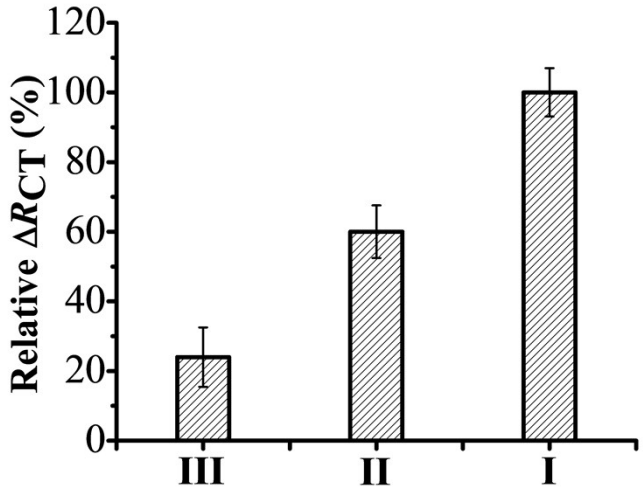

Fig.S2. Bar graph showing the relative change in ΔR_{CT} values for ODN **I-III** films obtained from EIS measurements before and after addition of Hg(II).

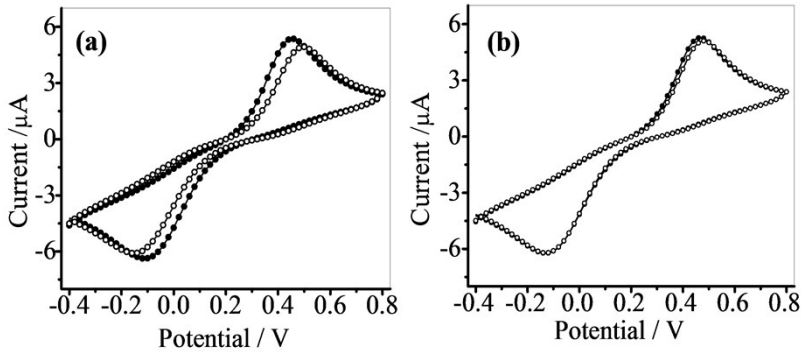

Fig.S3. Cyclic voltammograms for ODN films (a) ODN **II** and (b) ODN **III**; before (●) and after (○) incubation in buffer solution (20 mM MOPS buffer solution containing 150 mM $NaClO_4$ at pH = 7.4) containing 10 μM Hg(II).

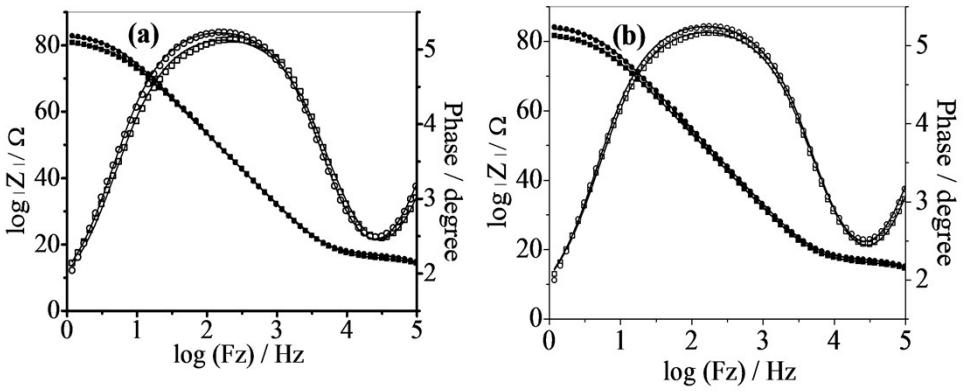

Fig.S4. Bode plots showing the variation of impedance magnitude (black) and phase angle (white) with respect to logarithm of frequency before (●) and after (■) incubation of ODN modified gold electrodes for (a) ODN **II**; and (b) ODN **III** in 10 μM Hg(II) solution. The solid lines represent impedance curves calculated by using the circuit model shown in the inset in Fig.1a. The measurements were carried out in 20 mM MOPS buffer solution containing 150 mM $NaClO_4$ at pH = 7.4.

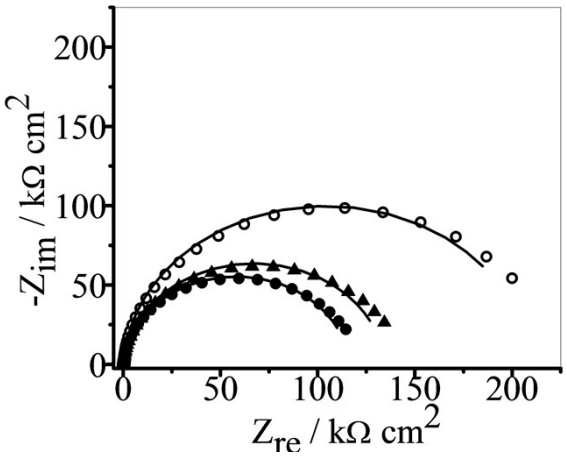

Fig.S5. Representative Nyquist plot of ODN **I** before (●) and after (○) incubating with Hg(II) and mixture of Hg(II) and cysteine (▲). The measurements were carried out in 20 mM MOPS buffer solution containing 150 mM $NaClO_4$ at pH = 7.4. Experimental data is shown as scatters with calculated solid lines fitted using the equivalent circuit as shown in the inset of Fig.1(a).

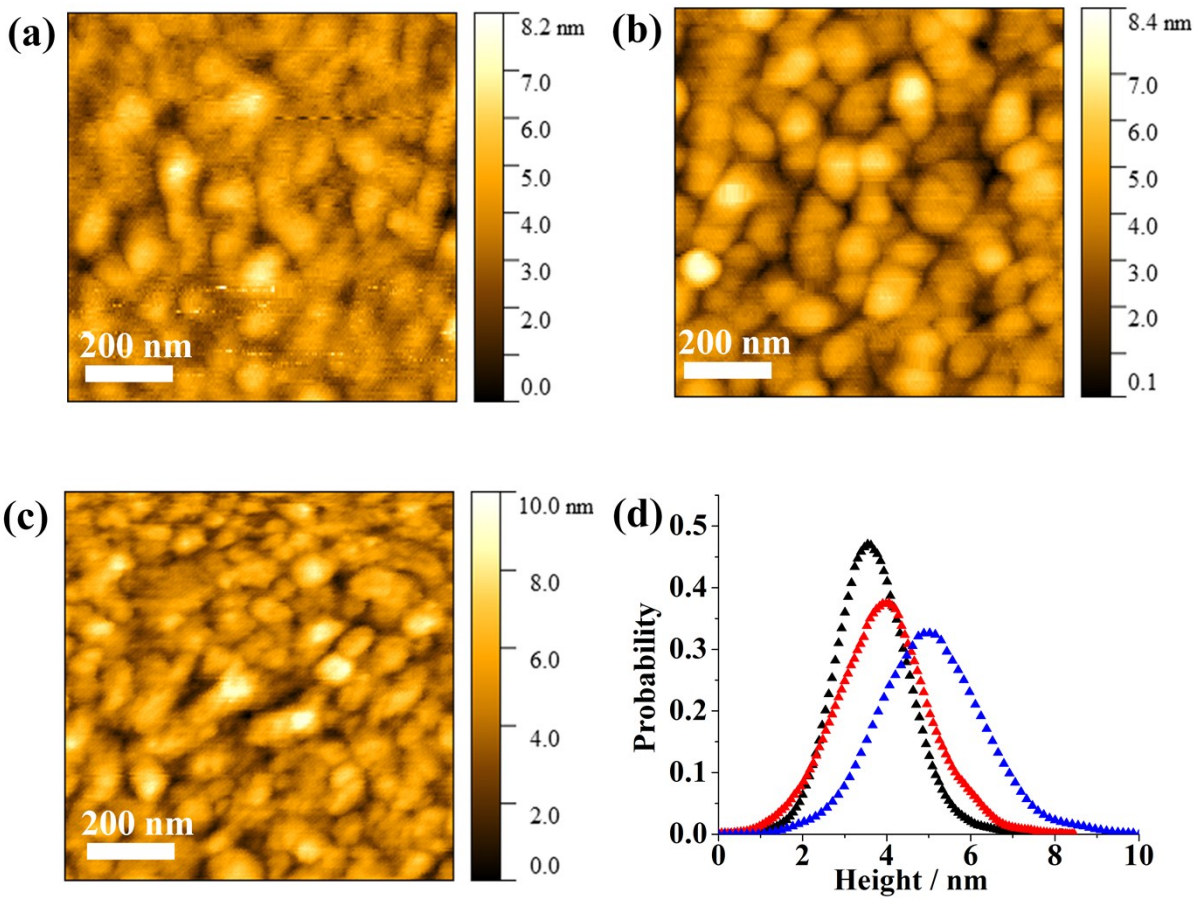

Fig. S6 (a) Atomic force microscopy images of bare gold surface and surfaces modified with ODN **I** (b) before and (c) after expose to the 10 μM Hg(II). The images were obtained using contact mode AFM under ambient condition. Plot (d) shows the height distributions corresponding to bare gold (black), ODN **I** modified (red) and ODN **I**-Hg(II) modified (blue) surfaces.

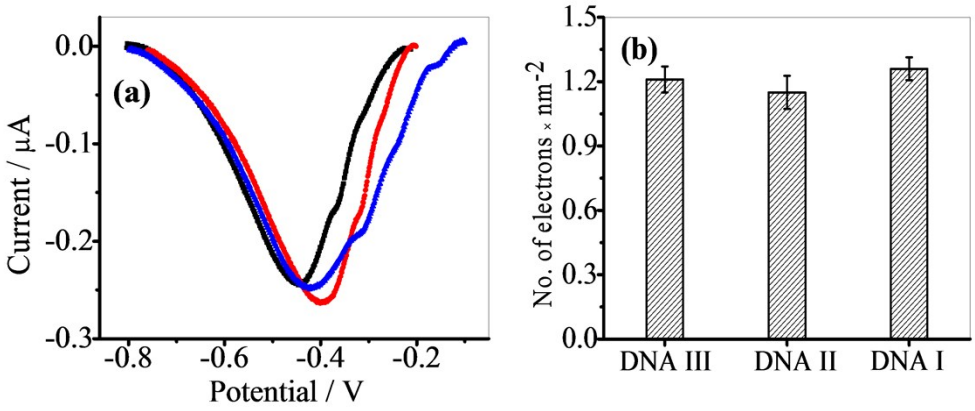

Fig.S7. (a) Reductive desorption peaks for ODN **I** (black), ODN **II** (red) and ODN **III** (blue) recorded in 0.5 M KOH at a rate of 0.05 Vs^{-1}; and (b) Electron density of the reductive desorption which was obtained by integrating the desorption peaks over time. The electron density of the individual ODNs do not appear to be significantly different.

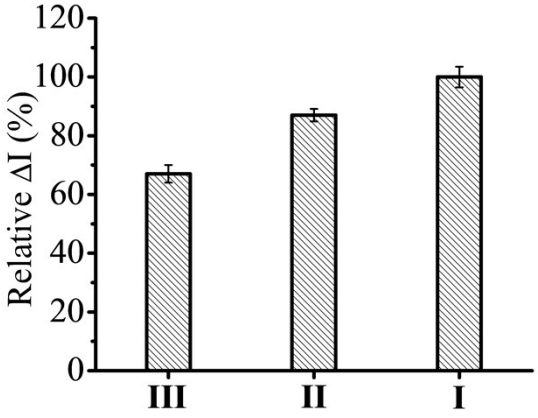

Fig.S8. Bar graph showing the relative change in ΔI values for ODN **I-III** films obtained from SECM measurements before and after addition of Hg(II).

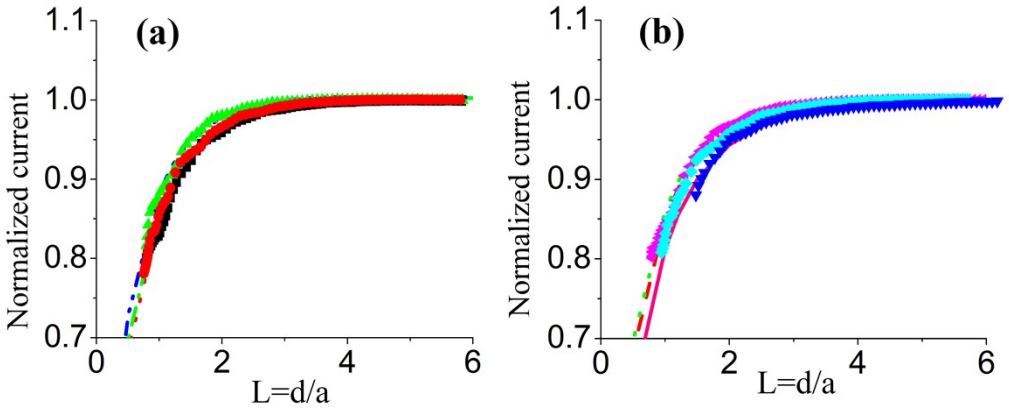

Fig.S9. (a) Normalized approach curves for ODN **I-III** microarrays before; **I**(■), **II**(●) and **III**(▲) and after exposure to Hg(II), **I**(▼), **II**(◆) and **III**(◀). Experimental data is shown as scatters and the dashed lines are approach curves obtained by using the COMSOL Multiphysics simulation. The measured current was normalized using the measured tip steady-state current at an infinite distance from the substrate. The normalized distance (L) is the ratio of the tip/substrate separation (d/a) to the tip radius.

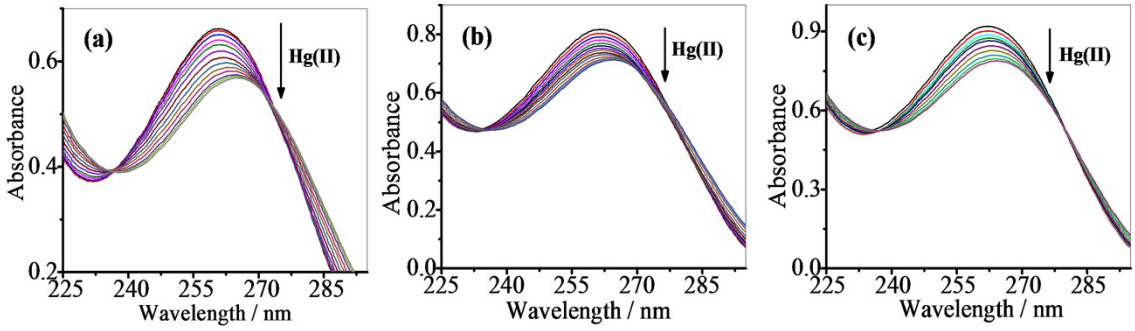

Fig.S10. UV spectra of ODNs at 5.0 μM concentration in the absence and presence of increasing concentration of Hg (II): (a) ODN **I**; (b) ODN **II;** and (c) ODN **III**. The measurements were performed in 20 mM MOPS buffer solution containing 150 mM $NaClO_4$ at pH = 7.4.

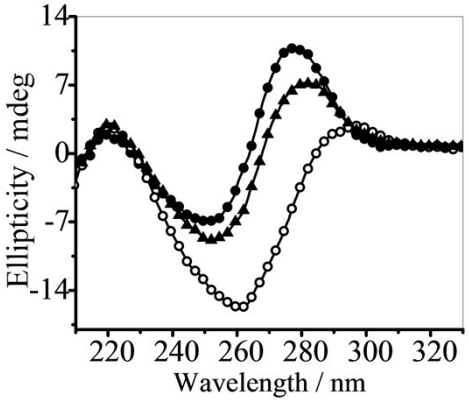

Fig.S11. CD spectra of ODN **I** (●) at 5.0 μM concentration in the presence of Hg(II) (○) and mixture of Hg(II) and cysteine (▲). The measurements were performed in 20 mM MOPS buffer solution containing 150 mM $NaClO_4$ at pH = 7.4.

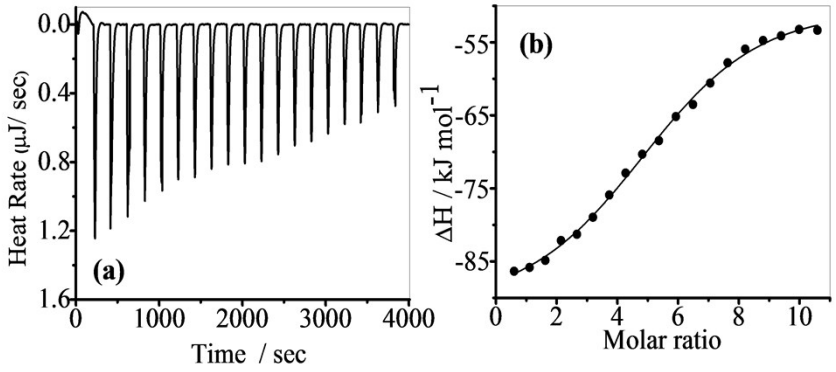

Fig.S12. (a) ITC raw data for titration of ODN **I** with Hg(II); and (b) ITC profile of the interaction between Hg(II) and ODN **I** in 20 mM MOPS buffer solution containing 150 mM $NaClO_4$ at pH = 7.4 and 298.15 K.

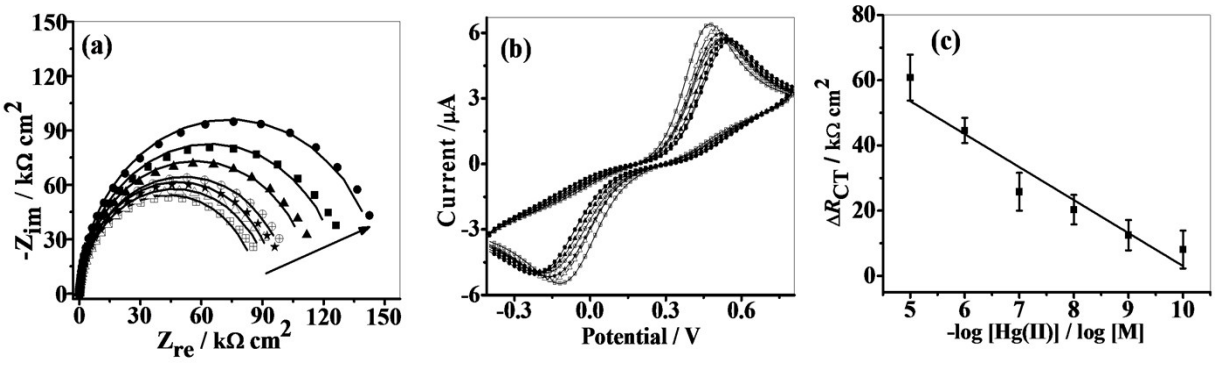

Fig. S13. (a) Representative Nyquist plots ($-Z_{im}$ *vs.* Z_{re}) for films of ODN **I** before (⊞) and after incubating in different spiked concentrations of Hg(II) in Ontario lake water ranging from 10^{-5} (●), 10^{-6} (■), 10^{-7} (▲) and 10^{-8} (⊕), 10^{-9} (★) and 10^{-10} M (△). Experimental data is shown as scatters with calculated solid lines fitted using the equivalent circuit as shown in the inset of Fig.1(a). The measurement was carried out in an electrolyte containing 2.0 mM $[Fe(CN)_6]^{3-/4-}$ as the redox probe in similar conditions; (b) Cyclic voltammograms for films of ODN **I** before (⊞) and after incubating in different spiked concentrations of Hg(II) in Ontario lake water ranging from 10^{-5} (●), 10^{-6} (■), 10^{-7} (▲) and 10^{-8} (⊕), 10^{-9} (★) and 10^{-10} M (△); and (c) Relationship between ΔR_{CT} and logarithmic concentration of Hg(II). Error bars indicates standard deviations from three replicated experiments.

Table S1. Equivalent circuit element values for ODN **I** films before and after binding to different spiked concentrations of Hg(II) in Ontario lake water ranging from 10^{-5} to 10^{-10} M. *represents change in charge transfer resistance (R_{CT}) values of ODN **I** before and after incubating with different concentrations of Hg(II). ΔR_{CT} = R_{CT} (after Hg(II) immersion) - R_{CT} (before Hg(II) immersion).

	R_s ($\Omega.cm^2$)	$C_{monolayer}$ (F/cm^2)	R_{CT} ($k\Omega.cm^2$)	R_x ($\Omega.cm^2$)	CPE (F/cm^2)	n	ΔR_{CT}* ($k\Omega.cm^2$)
ODN I	5.6	7.35E-09	86.80	175.4	2.69E-07	0.97	-
1×10^{-5} M	5.5	5.35E-09	149.3	179.5	2.10E-07	0.95	62.5
1×10^{-6} M	5.9	5.88E-09	131.4	180.4	2.18E-07	0.96	44.6
1×10^{-7} M	5.7	6.02E-09	112.6	179.2	2.35E-07	0.98	25.8
1×10^{-8} M	5.5	6.55E-09	108.1	173.6	2.54E-07	0.95	21.3
1×10^{-9} M	5.8	6.79E-09	98.20	180.5	2.65E-07	0.98	11.4
1×10^{-10} M	5.9	7.15E-09	94.15	168.2	2.85E-07	0.97	7.35

References

(1) A. Kamal, Z. She, R. Sharma, H. B. Kraatz, *Electrochim. Acta,* 2017, **243,** 44-52.

(2) D. Y. Petrovykh, H. K. Suda, M. J. Tarlov, L. J. Whitman, *Langmuir,* 2004, **20**, 429-440.

(3) C. J. Powell, A. Jablonski, *Surf. Interface Anal.,* 2002, **33,** 211-229.