Supplementary Material for Chemical Communications

An Ultrasensitive Nucleic Acid Biosensor Based on the Catalytic Oxidation of Guanine by a Novel Redox Threading Intercalator

Natalia C. Tansil, Fang, Xie, Hong Xie and Zhiqiang Gao*

Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, Singapore 138669, Singapore.

SUPPORTING INFORMATION

EXPERIMENTAL DETAILS

Chemicals. 1(3-aminopropyl)-imidazole (98%, AI) and 1,4,5,8-naphthalene tetracarboxylic dianhydride (>95%) were purchased from Sigma-Aldrich (St Louis, MO, USA). $Ru(bpy)_2Cl_2$ (99%) was from Avocado Research Chemicals Ltd (Leysham, Lancester, UK). All other reagents were obtained from Sigma-Aldrich and used without further purification. CPs used in this work were custom-made by Alpha-DNA (Montreal, Canada) and all other oligonucleotides were custom-made by 1st Base Pte Ltd (Singapore). A 10 mM Tris-HCl–1.0 mM EDTA–0.10 M NaCl buffer solution (TE) was used as hybridization buffer. A phosphate-buffer saline (PBS, pH 7.4), consisted of 0.15 M NaCl and 20 mM phosphate buffer, was used as the supporting electrolyte. Oligonucleotide sequences for TP53 detections were as follows:

5'-HS-$(CH_2)_6$-T_6GTGACACGCTTCCCTGGATT-3' (capture probe),

5'-HS-$(CH3)_6$-T_6TATGGCGGGAGGTAGACTGA-3' (capture probe),

5'-HS-$(CH_2)_6$-T_6GT**T**ACACGCTTCCCTGGATT -3'(one-base-mismatched),

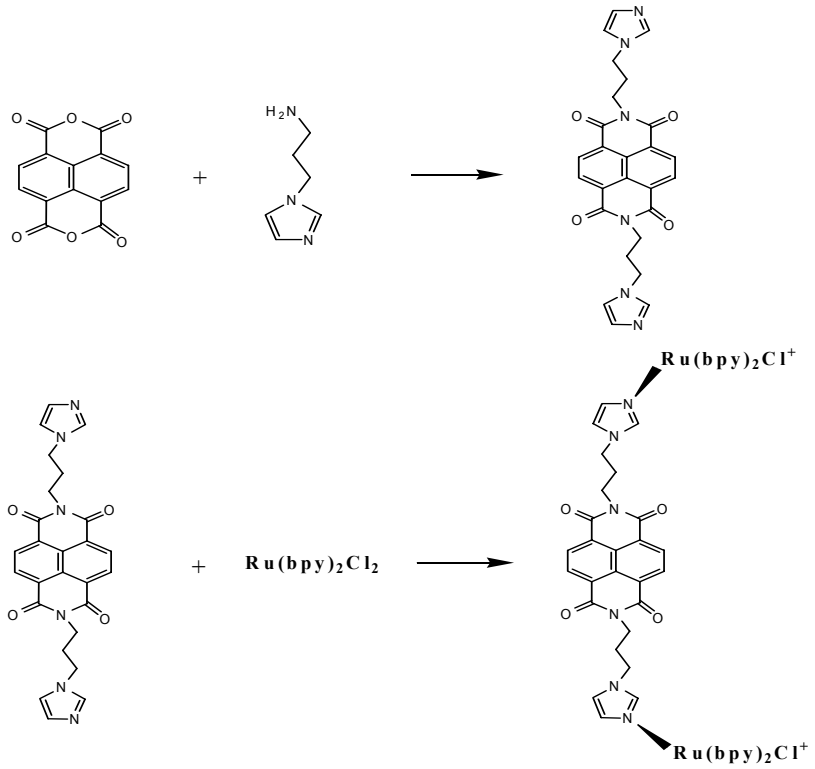
5'-HS-$(CH_2)_6$-T_{12}TATGGCGGGAGGTAG**G**CTGA-3'(one-base-mismatched),

5'-HS-(CH3)6-T_{12}CCTCTCGCGAGTCAACAGAA-3' (control).

Apparatus. Electrochemical experiments were carried out using a CH Instruments model 660A electrochemical workstation coupled with a low current module (CH Instruments, Austin, TX). The three-electrode system consisted of a 3.0-mm-diameter gold working electrode, a non-leak miniature Ag/AgCl reference electrode (Cypress Systems, Lawrence, KS), and a platinum wire counter electrode. To avoid the spreading of the sample droplet beyond the 2.0-mm diameter working area, a patterned hydrophobic film was applied to the gold electrode after the immobilization of the CP. All potentials reported in this work were referred to the Ag/AgCl electrode. UV-visible spectra were recorded on an V-570 UV/VIS/NIR spectrophotometer (Jasco, Corp., Japan) Mass spectrometric experiments were performed with a Finnigan/MAT LCQ Mass Spectrometer (ThermoFinnigan, San Jose, CA). All spectra were recorded at room temperature unless otherwise noted.

Synthesis of N,N'-bis[1(3-propyl)-imidazole]-1,4,5,8-naphthalene diimide (PIND) grafted with $Ru(bpy)_2Cl_2$ (PIND-Ru). The synthesis of PIND-Ru is outlined in Scheme 1. PIND was prepared following a general procedure for the synthesis of ND.[1,2] Briefly, to a magnetically stirred mixture of 3.0 ml of AI and 3.0 ml of tetrahydrofuran was slowly added 0.30 g of 1,4,5,8-naphthalene tetracarboxylic dianhydride. The rate of addition was controlled so that there was little clogging. The reaction mixture was refluxed for 24 h and then cooled to room temperature. Next, it was dispersed in 10 ml of acetone/water (3/1) mixture and then poured into 500 ml of rapidly stirred anhydrous ether to precipitate the compound. The precipitate was collected by suction filtration through a fine fritted funnel and washed briefly with ethanol. Purification was performed by crystallization from chloroform/ethanol (1/1 by volume) and dried under vacuum at 40ºC overnight to give 0.46 g of yellow crystals (yield 85%). 1H NMR (300 MHz $CDCl_3$) δ 8.76 (4H), 7.54 (2H), 7.26 (2H), 4.27 (4H), 4.12(4H), 2.31 (4H) and 1.83 9(2H) (Figure 1). Mass spectrometric tests on PIND using electron-spray ionization mass spectrometry (ESI-MS) showed predominant peaks at m/z 483.3 and 242.3, corresponding to $PIND+H^+$, (and $(PIND+2H^+)/2$ (Figure 2), which are in good agreement with the

molecular weights of the desired compounds. Since mono-grafted PIND was not observed in the ESI-MS spectrum, we can rule out any incomplete grafting of PIND.



Scheme 1. Synthetic route to PIND-Ru intercalator

PIND-Ru was synthesized in a single-step ligand-exchange reaction. To a solution of $Ru(bpy)_2Cl_2$ (0.55 mmol) in 8.0 ml freshly-distilled ethylene glycol was added PIND (0.25 mmol) in small portions over 10 min; and the resulting mixture was refluxed for 30 - 40 min. The completion of the ligand-exchange reaction was monitored by cyclic voltammetry. The purple reaction mixture was then poured slowly into 100 ml of rapid stirred ethanol saturated with KCl. The precipitate was collected by suction filtration through a fine fritted funnel. The crude product was washed with PBS, dissolved in 3.0 - 5.0 ml of ethanol and precipitated again from KCl saturated ethanol. The precipitate was further purified by crystallization from ethanol giving the pure product in 78% yield. The product showed a single pair of reversible redox peaks at the gold electrode with an $E_{1/2}$ of 0.63 V in PBS. To ensure a complete double ligand-exchange at the two imidazole termini of PIND, slight excess of $Ru(bpy)_2$ (10-25%) is required.

Immobilization of CP on gold electrode. Prior to capture probe adsorption, a gold electrode was exposed to oxygen plasma for 5-10 min and then immersed immediately in absolute ethanol for 20 min to reduce the oxide layer. CP monolayer was adsorbed by immersing the gold electrode in a PBS solution of 100 μg/ml CP for 16-24 h. After adsorption, the electrode was copiously rinsed with and soaked in PBS for 20 min, rinsed again, and blown dry with a stream of air. The surface density of CP, assessed electrochemically by the use of cationic redox probe according to the procedure proposed by Steel,[3] was found to be in the range of $1.13\text{-}1.30\text{x}10^{-11}$ mol/cm^2. To minimize the non-DNA related PIND-Ru uptake and improve the quality and stability of the CP monolayer, the CP-coated gold electrode was immersed in an ethanolic solution of 2.0 mg/ml 1-mercaptododecane (MD) for 4-6 h. Unreacted MD molecules were rinsed off and the electrode was washed by immersion in a stirred ethanol for 10 min followed by thorough rinsing with ethanol and water. The electrode was ready after air-dry.

Hybridization and detection. The hybridization of a target DNA and its electrochemical detection were carried out in three steps. First, an aliquot of hybridization solution containing the target DNA was uniformly spread onto the biosensor and the biosensor was placed in a moisture-saturated environmental chamber maintained at 60ºC (low stringency 27ºC below the salt-adjusted melting temperature) for 30 min. It was then rinsed thoroughly with a blank hybridization solution at 60ºC and incubated at 35ºC for 10 min with a 5.0 μl aliquot of 100 μg/ml of PIND-Ru in the hybridization solution. PIND-Ru was attached to the hybridized target DNA via threading intercalation. After the biosensor was air-cooled and held at room temperature for 10 min, a thorough rinsing with NaCl-saturated phosphate buffer (pH7.4) containing 10% ethanol was conducted. The electrocatalytic oxidation current was measured voltammetrically. At low DNA concentrations, smoothing was applied after each measurement to remove random noise and electromagnetic interference. Prior to hybridization, the mRNA mixture was denatured at 70°C for 10 min. To minimize the effect of

RNases on the stability of mRNA, all solutions were treated with diethyl pyrocarbonate and surfaces were decontaminated with RNaseZap (Ambion, TX).

FORMATION OF PIND-Ru

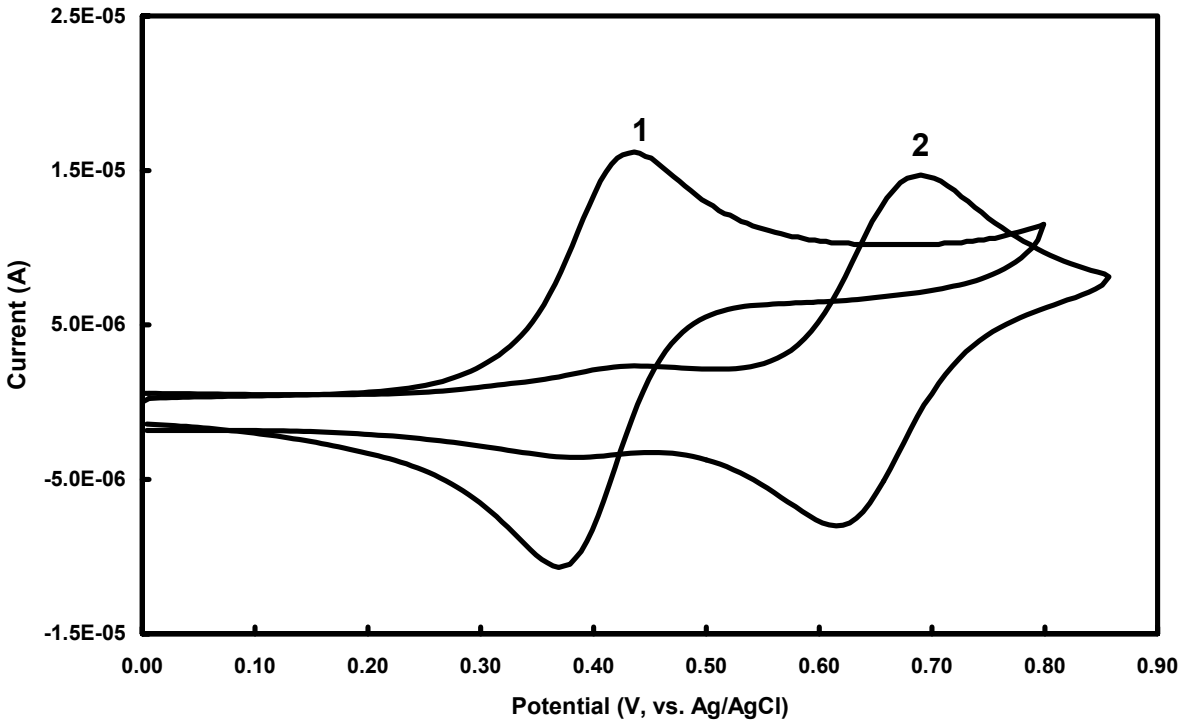


Figure 1 Cyclic voltammograms of (1) $Ru(bpy)_2Cl_2$ and (2) $Ru(bpy)_2Cl_2$ after 30 min of refluxing with PIND in ethylene glycol. Supporting electrolyte for (3) PBS, potential scan rate 100 mV/s.

The formation of the electroacticve PIND-Ru intercalator can be conveniently monitored by cyclic voltammetry. During reflux in ethylene glycol, cyclic voltammetric tests were conducted every 5 min. Figure 1 shows two typical voltammograms obtained in the first 30 min. As can be seen in trace 1 in Figure 1, before adding PIND to $Ru(bpy)_2Cl_2$, one pair of reversible voltammetric peaks centered at 0.40 V were obtained, corresponding to the well-known redox process of $Ru(bpy)_2Cl_2$. Upon adding PIND, a new pair of voltammetric peaks appeared at 0.63 V, indicating the formation of PIND-Ru (Figure 1 trace 2). Both electron transfer processes are clearly resolved and exhibit all the characteristics of reversible processes, except for the slightly larger peak-to-peak potential separation

that is mainly due to a higher iR drop of the reaction medium. The intensities of the voltammetric peaks at 0.63 V increased gradually with reaction time, while those at 0.40 V diminished gradually. Both of the redox pairs reached a steady-state after 30-40 min of refluxing. The minute voltammetric peaks at 0.40 V are indicative of the excess amount of $Ru(bpy)_2Cl_2$. After separation and purification, voltammetric tests of the thus purified PIND-Ru showed only one pair of voltammetric peaks implying that the purification process is very effective (Figure 2).

ELECTROCHEMISTRY OF PIND-Ru

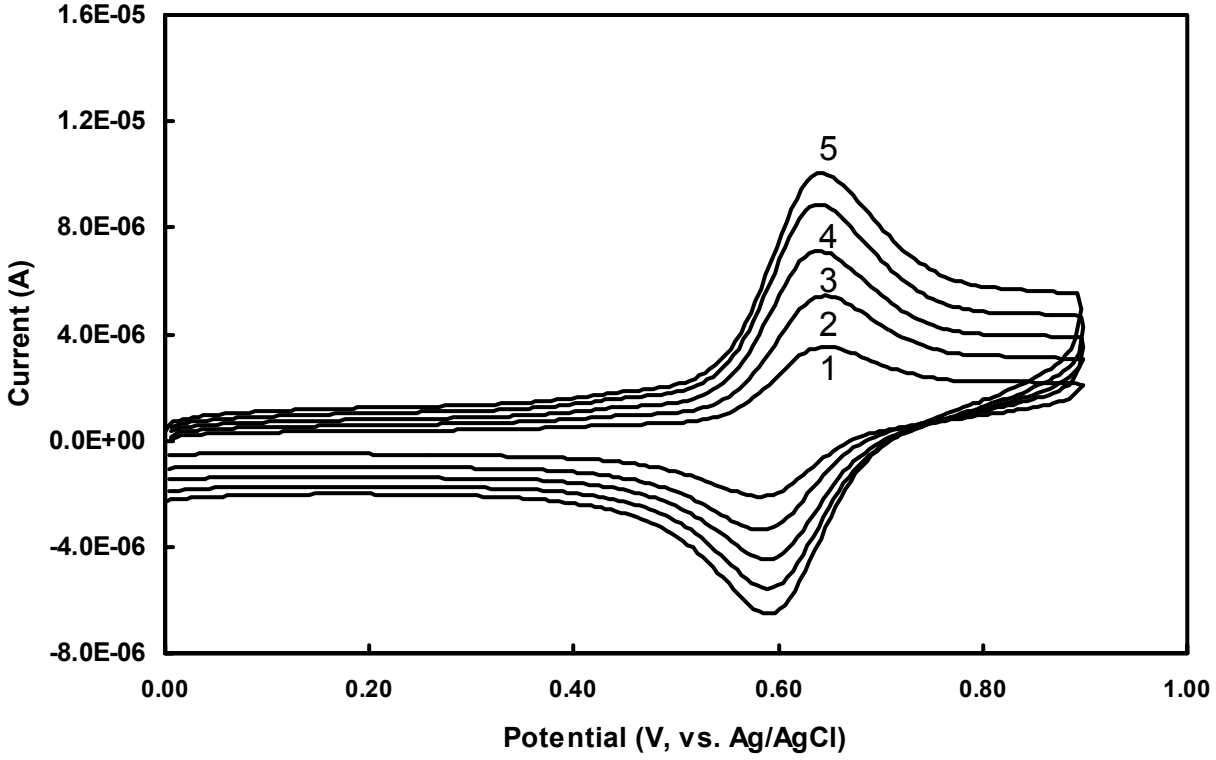


Figure 2. Cyclic voltammograms of the purified PIND-Ru in PBS. Potential scan rate from the innermost to the outmost voltammograms were (1) 100, (2) 200, (3) 300, (4) 400 and (5) 500 mV/s.

As illustrated Figure 2, PIND-Ru exhibited exactly as expected for a highly reversible redox couple in solution. Little change was observed after numerous repetitive potential cycling between 0.0 and +0.90V, revealing good stability of PIND-Ru in solution. At slow scan rates, <500 mV/s, a typical diffusion-controlled voltammogram was recorded as expected for a one-electron exchange system exhibiting an

ideal Nernstian behavior: the peak current is proportional to the square root of the potential scan rate, the peak-to-peak potential separation is very close to the theoretical value of 59 mV, and potential scan rate independent. Such results ascertain that all of the ruthenium redox centers proceed to reversible heterogeneous electron transfer.

REFERENCES

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