Supporting Information

Label-free Electrochemical Detection of Adenosine Based on Electron Transfer from Guanine Bases of Its Aptamer

Jinheung Kim,* Min Sun Choi, In Young Kim, Qiong Wu

[*] I. Y. Kim, M. S. Choi, Q. Wu, Prof. Dr. J. Kim
Department of Chemistry and Nano Science
Ewha Womans University
Seoul 120-750, Korea
Fax: (+82) 2-3277-3419
E-mail: jinheung@ewha.ac.kr

[**] This research was supported by the SRC/ERC program of MOST/KOSEF (R11-2005-008-000-0) and Seoul R&BD program (10816), and the Korea Research Foundation (KRF-2005-015-C00225), BK21 (IYK, MSC, QW).

Experimental Section

Materials and Instrumentation. Water was purified with a MilliQ purification system. $[Ru(bpy)_3]Cl_2$ was purchased from Aldrich. All oligonucleotides were purchased from JenoTec Inc. (Deajeon, Korea) and were purified by HPLC. Adenosine and other nucleosides were purchased from Aldrich. ITO electrodes were purchased from Delta Technologies, Inc (USA). The Ag/AgCl reference electrode was purchased from Cypress, Inc. Oligonucleotide concentrations were determined spectrophotometrically monitoring the absorbance at 260 nm, ε= 6600 M^{-1} cm^{-1} on a Hewlett-Packard 8452A diode-array spectrometer.

The electrode cleaned by sequential 10 min sonications in Alkonox, 95% ethanol, water twice, and finally the desired buffer. Voltammetry was collected using a CH instrument potentiostat (CHI630C) with a single-compartment voltammetric cell equipped with an indium tin oxide (ITO) working electrode (area = 0.32 cm^2), a Pt wire counter electrode, and an Ag/AgCl reference electrode.

Electrochemical Adenosine Detection. In a typical experiment, a sample containing 50 uM metal complex and 40 uM oligonucleotide dissolved in buffered aqueous solution containing 50 mM Na/phosphate buffer (pH = 6.8) was scanned at 25 mV/s from 0.0 V to 1.3 V. A freshly cleaned ITO electrode was used for each experiment, and a background scan of buffer alone was collected for each electrode and subtracted from subsequent scans.

Preparation of AuNPs on ITO electrodes. ITO electrodes were successively cleaned with Alconox(4g/L) for 15 min, 2-propanol for 15 min, and Milli-Q water twice by sonication, and then dried at 50°C. The cleaned substrates were pretreated in a mixture of 5:1:1 H_2O/H_2O_2 (30%)/NH_4OH (30%) (v/v/v) at 70 °C for 90 min to ensure the formation of hydroxyl groups on the surface. The substrates were then washed with copious amount of water and dried at 50°C. The pretreated ITO electrodes were immersed in an ethanol solution containing 2% 3-aminopropyltriethoxysilane (APTES, v/v) for 12 h to induce the formation of the aminosilane monolayer. It was then washed thoroughly with ethanol (three times), and dried at room temperature. The amine-functionalized ITO electrodes were dipped in 1 mM $HAuCl_4$ (pH 3.13) for 30 min. The $AuCl_4^-$ ions are electrostatically bound to protonated amine groups. After thorough rinsing with water, the electrostatically bound $AuCl_4^-$ ions were

reduced in an aqueous solution containing 0.5 mM ascorbic acid for 2 h. The AuNP-loaded ITO electrodes were washed with water and dried at 50 °C.

Electrochemical Detection on AuNP-ITO Electrodes. In a typical experiment, The AuNP-loaded ITO electrodes were incuvated with 20 uM **1** for 30 min at RT and washed with Na/phosphate buffer. Then, the solution containing 50 uM $Ru(bpy)_3^{2+}$ in 50 mM Na/phosphate buffer was loaded on the AuNP-ITO electrode and was scanned at 25 mV/s from 0.0 V to 1.3 V. A background scan of buffer alone was collected for each electrode and subtracted from subsequent scans.

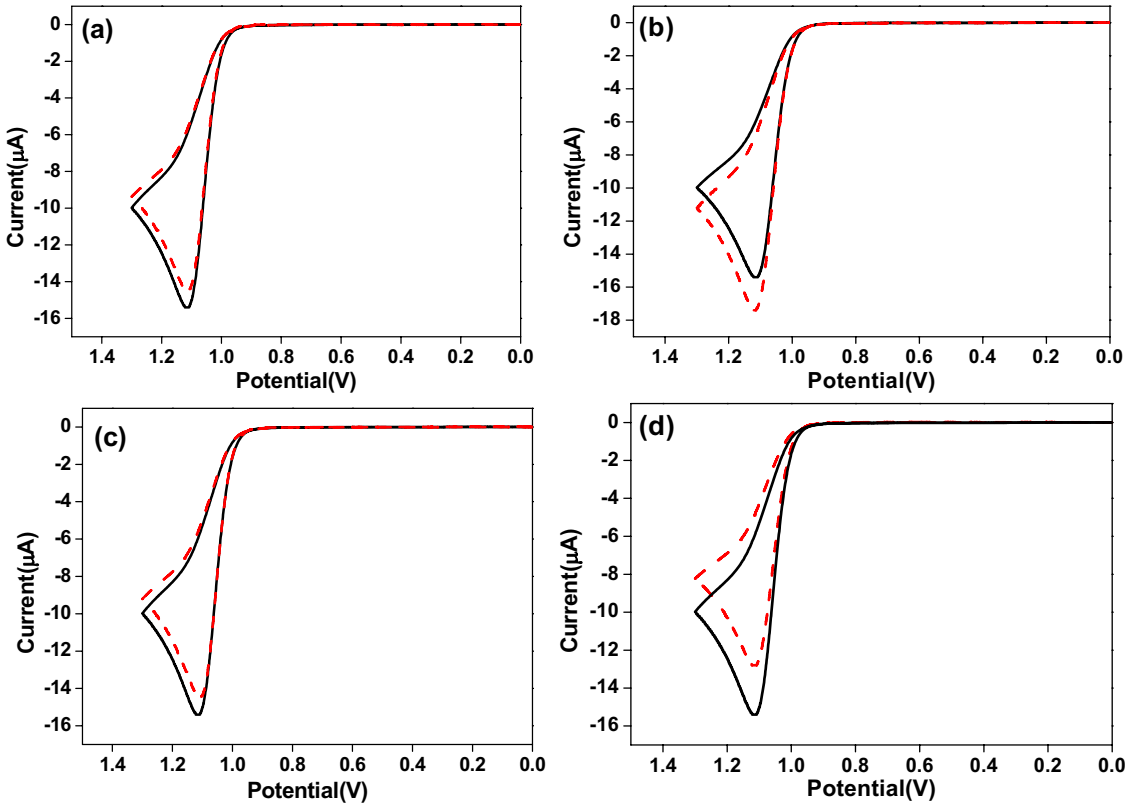

Figure S1. Cyclic voltammograms of 50 uM $Ru(bpy)_3^{2+}$ with **1** in the presence of (a) cytidine , (b) inosine, (c) uridine at ITO working electrode. The dotted line in each Figure indicates the CV of $Ru(bpy)_3^{2+}$ with **1** only. (d) Cyclic voltammograms of 50 uM $Ru(bpy)_3^{2+}$ and **2** with (dotted line) and without (solid line) adenosine. The scan rate is 25 mV/s, and all potentials are versus Ag/AgCl.

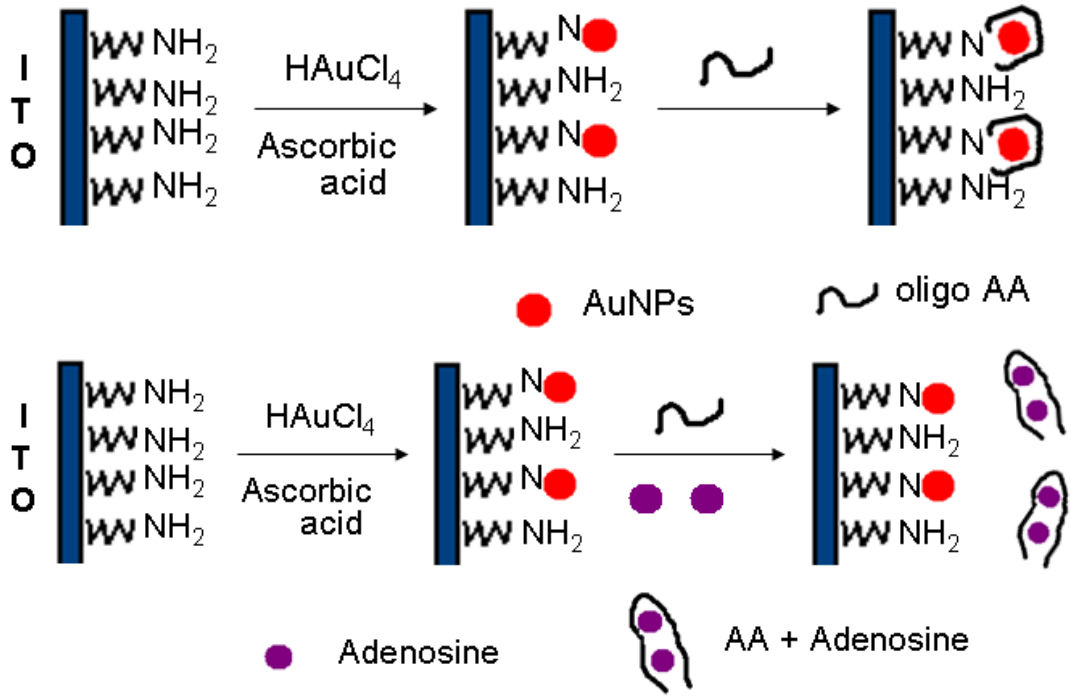

Figure S2. Schematic representation of the formation of AuNP on an APTES-modified ITO electrode, and an electrochemical aptasensor for detecting adenosine.

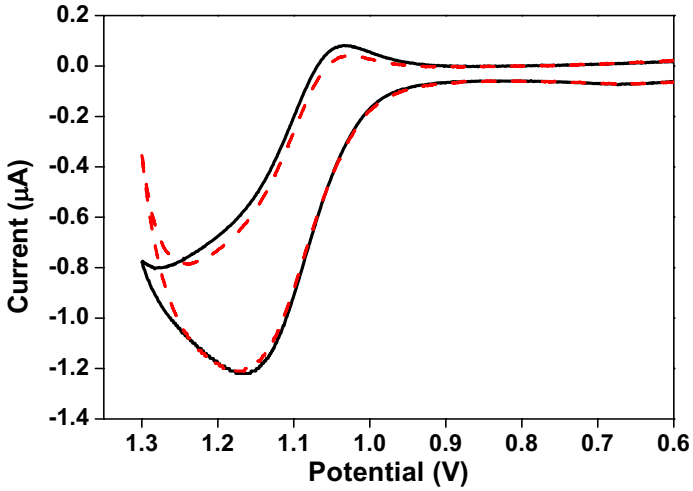

Figure S3. Cyclic voltammograms of 50 uM $Ru(bpy)_3^{2+}$ obtained with the amine-functionailzed ITO electrode treated with (dotted line) and without (solid line) treatment of **1**. The scan rate is 25 mV/s, and all potentials are versus Ag/AgCl.