

Supporting Information

Aptamer Conformational Switch as Sensitive Electrochemical Biosensor for Potassium Ion Recognition

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Experimental Section

Materials. 2-mercaptoethanol (2- ME), was purchased from Fluka and N-(2-hydroxyethyl) piperazine-N'-2-ethanesulfonic acid (HEPES), ethyleneglycol bis(β -aminoethyl ether)-N,N,N,'N'-tetraacetic acid (EGTA), Ammonium hexacyanoferrate(II) hydrate, Tris(tetrabutylammonium) hexacyanoferrate(III), NaCl, KCl, NaOH, MgCl₂, and CaCl₂ were from Aldrich Chemicals. All reagents were used without further purification. All solutions were prepared using Milli-Q water. The aptamer (5'-GGTTGGTGTGGTTGG-3'), HPLC purified and lyophilized modified with a SH at the 3' end was provided by Cultek, S.L. (Eurogentec, S.A.). Ferrocene-Labeled aptamer was produced by incorporation of a ferrocene label to the 5'-ter of the aptamer by the EDC-NHS method as described previously.^a Buffer solution were prepared from 10 mM HEPES adjusted with 1.0 M NaOH to pH 8.0. EGTA was prepared in HEPES and the pH of the solution was adjusted at 8.0 by adding 1.0 NaOH.

Electrochemical Measurements. All electrochemical measurements were performed with Autolab model PGSTAT 12 potentiostat/galvanostat (Eco Chemie), controlled by GPES4 and FRA software, which were used for acquisition and analysis of the electrochemical data. A conventional three-electrode system, consisting of a bare or modified gold

^a Schuhmann, W.; Ohara, T. J.; Schmidt, H. L.; Heller, A. *J. Am. Chem. Soc.* **1991**, *113*, 1394-1397.

working electrode, a platinum wire auxiliary electrode, and an Ag/AgCl/3.0 M NaCl reference electrode (from Bioanalytical System Inc., Lafayette, IN) was used. All potentials are reported versus Ag-AgCl reference at room temperature. All potentials are reported versus Ag-AgCl reference at room temperature. Square-wave voltammetry was with step potential of 4 mV, amplitude of 50 mV, and frequency of 120 Hz. Baseline correction of the resulting voltammogram was performed using the "moving average" mode. Electrochemical impedance measurements were performed in the presence of equimolar concentrations, 1.0 mM ($[\text{Fe}(\text{CN})_6]^{3-}$, $[\text{Fe}(\text{CN})_6]^{4-}$) as redox probe, at the formal redox potential, using a sinusoidal ac potential perturbation of 5 mV, in the frequency range 100 kHz to 10 mHz, and readings were taken at 20 discrete frequencies per decade. The impedance spectra were plotted in the form of Nyquist plots.

Procedures. The electrodes were polished with 1.0 μm alumina, followed by 0.3 and 0.05 μm alumina slurry on microcloth pads (Buehler, Lake Bluff, IL). After removal of the trace alumina from the surface by rinsing with Milli-Q water and brief cleaning in an ultrasonic bath, the electrodes were cleaned further by electrochemical etching in 0.05 M H_2SO_4 by cycling the electrode potential between -0.3 and +1.5 V until a reproducible cyclic voltammogram was obtained. The aptamer-modified gold electrodes were prepared using the previously described procedure.^b The biosensing protocol at the aptamer-modified electrode consisted of three steps: preconcentration, measurement and regeneration. In the preconcentration step, carried out in an open circuit, the aptasensor was immersed in 2.0 mL of a magnetically stirred solution of metal cation HEPES buffer solution (pH 8.0, 0.01M) for 30 s. The sensor was then removed from the preconcentration solution, washed with water, and transferred to a cell containing the supporting electrolyte (10 mM HEPES buffer). Before and between every electrochemical assay the modified electrode was immersed for 90 s in a stirred solution of 0.1 M HCl. To ensure that the aptasensor was completely regenerated, the aptasensor was placed in HEPES solution, and a square wave voltammogram recorded to ensure the lack of an electrochemical signal, thus confirming regeneration.

^b Radi, A.E.; Sanchez, J. L. A.; Baldrich, E.; O'Sullivan, C. K. *J. Am. Chem. Soc.* **2006**, *128*, 117-124.