Supplementary Material (ESI) for Chemical Communications

Aptamer biosensor for label-free impedance spectroscopy detection of proteins based on recognition-induced switching of the surface charge

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I. Apparatus

Impedance spectroscopy experiments were performed using the μAutolab III analysis system with GPES and FRA 4.9 software (Eco Chemie). The detection was carried out in a 1.0 mL electrochemical cell containing a working electrode comprised of indium tin oxide (ITO) coated glass (Delta Technologies, Stillwater, MN**),** a Ag/AgCl reference electrode (CH Instruments, Austin, TX), and a platinum wire as a counter electrode. The ITO electrodes were patterned using standard photolithographic and wet chemical etching processes to define a disk with a 3 mm diameter.

II. Reagents

All stock and buffer solutions were prepared using autoclaved double-deionized water (18.2 MΩ). Acetate buffer (3M, pH 5.2), sulfuric acid, phosphate buffer, Tris-HCl, NaCl, $MgCl_2$, Tween 20, potassium ferricyanide, potassium ferrocyanide, potassium chloride, 1-ethyl-3-(3-dimethyl aminopropil)carbodiimide (EDAC), N-hydroxy-succinimide (NHS), ethanolamine-HCl, 2-aminobenzoic acid (ABA), streptavidin, thrombin, lysozyme, cytochrome C, bovine serum albumin, acetone and 2-propanol were purchased from Sigma (St. Louis, MO), while alconox was provided by VWR Scientific (West Chester, PA). All chemicals were of analytical reagent grade. All experiments were conducted at room temperature (unless specified otherwise).

The compositions of the different buffers used for the surface modification and assay were as the following:

- **Streptavidin attachment buffer**: 10 mM acetate buffer (pH 5.2).
- **Immobilization-Binding (I-B) buffer**: 20 mM Tris-HCl/0.1M NaCl/5 mM $MgCl_2$ (pH 7.5).
- **Washing buffer**: 20 mM Tris-HCl/0.1 M NaCl/5 mM $MgCl_2$/1.0 % (v/v) Tween 20 (pH 7.5).

The anti-lysozyme biotinylated aptamer was purchased from Integrated DNA Technologies, Inc. (IDT, Coralville, IA) and has the following sequence: 5'-Biotin-ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3'

The anti-lysozyme biotinylated aptamer stock solution (1000 mg L^{-1}) was prepared with autoclaved water and was kept frozen.

III. Preparation of the Aptamer-Modified Electrode

III.1 Modification of the ITO Electrode Surface

The ITO electrode was first sonicated in acetone, followed by sonication in 1.0 % (w/w) detergent (Alconox), 2-propanol and distilled water, and was dried with a stream of nitrogen. The electrode was then modified by cycling its potential (8 cycles) between 0.0 and 1.0 V at 40 mV s^{-1} in a 1 M sulfuric acid solution containing 0.05 M ABA. Following the electropolymerization step, the ITO surface was activated with 100 mM NHS/400 mM EDAC in water. After 10 min, the electrode was washed with a 10 mM sodium acetate buffer (pH 5.2) for 20 min. The streptavidin covalent attachment step was carried out by dipping the modified electrode in a 10 mM acetate buffer (pH 5.2) solution containing 200 mg L^{-1} streptavidin for 12 hours at 4 °C. Then, the residual reacting groups were blocked

with 1 M ethanolamine-HCl (pH 8.5**)** for 20 min. The modified electrode surface was then rinsed with the washing buffer for additional 20 min.

III.2 Aptamer Immobilization

An 10 μL aliquot of an I-B buffer solution containing 200 mg L^{-1} of the biotinylated anti-lysozyme aptamer (previously heated up to 70 °C for 3 min and allowed to cool at room temperature) was placed onto the modified ITO electrode and kept at room temperature. Such pre-heating and cooling are essential for maintaining the structural robustness of the aptamer.[1] After 1 hour the solution was replaced for washing buffer during 20 min.

IV. Protein–Aptamer Association

A 10 μL sample droplet containing the target protein (in I-B buffer) was placed on the aptamer-functionalized ITO surface. The incubation reaction proceeded for 1 hour at room temperature. This binding event was followed by a 20 min washing step involving rinsing and placing the aptamer modified ITO electrode in the (Tris-HCl-Tween 20/NaCl/$MgCl_2$) washing buffer solution.

V. Faradaic Impedance Spectroscopy (FIS) Measurements.

FIS monitoring of the aptamer-protein interaction was performed in a 2 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) - 0.1 M KCl - 10 mM phosphate buffer (pH 7.0) solution. The spectra was recorded at a bias potential of +0.225 V (vs. Ag/AgCl), using a frequency range from 100 kHz to 10 mHz and an AC amplitude of 5 mV.

References:

1. R. Kirby, E. J. Cho, B. Gehrke, T. Bayer, Y.S. Park, D.P. Neikirk, J.T. McDevitt and A.D. Ellington, *Anal. Chem.* 2004,**76**, 4066.