SUPPLEMENTARY INFORMATION

for

An electrochemical immunosensor based on antibody-nanowire conjugates

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EXPERIMENTAL SECTION

Materials. Prostate-specific antigen and physiological human serum from AB donors were from Sigma-Aldrich Chemicals. All other chemicals used were of analytical grade and were obtained from Sigma-Aldrich Chemicals. Anti-PSA specific monoclonal antibodies were provided by Prof Eleftherios P. Diamandis (Mount Sinai Hospital, Toronto, Ontario, Canada). Antibody 8310 was used for immobilization and antibody 8311 was used for detection.

Apparatus. All electrochemical experiments were conducted with a BAS Epsilon potentiostat/galvanostat controlled with BAS Epsilon EC software (Bioanalytical Systems Inc, West Lafayette, USA). A three electrode configuration system was used, consisting of an antibody-modified gold nanowire electrode ensemble as working electrode, a platinum wire counter electrode and a Ag/AgCl reference electrode. Square wave voltammetry was performed at the potential range 0 to 800 mV (versus Ag/AgCl) in degassed 0.1M Tris-HNO_3 pH=9.8 supporting

electrolyte. The parameters for square wave voltammetry were: frequency 25 Hz, step 4 mV and amplitude 20 mV.

Preparation of gold nanowire electrode ensembles. Electrodes composed of three-dimensional gold nanowires were fabricated by templated electroless deposition within a polycarbonate membrane. Au nanowires about 200 nm in length and 10 nm in diameter were exposed from the membrane surface after oxygen plasma etching for 150 seconds. The scanning electron microscope (SEM) image (Scheme 1B) shows a representative field of the nanowires used in these experiments The nanowire electrodes generated for different experiments were scanned in a solution containing of 3 mM $Ru(NH_3)_6^{3+}$ in 25 mM sodium phosphate (pH 7), and 25 mM NaCl by CV to confirm that the working electrode area remained constant for different electrodes.

Preparation of antibody modified electrodes. 10 mM cystamine in water was applied for 16 h on gold nanowire electrodes in order to form a self-assembled monolayer (SAM). The SAM was allowed to react at room temperature with 2.5% glutaraldehyde in water for 1 h. The functionalized electrode was then reacted with 15 µl 10 µg/ ml capturing antibody 8310 in PBS for 1 h at 37^o C. The unreacted aldehydes were blocked with 2% bovine serum albumin (BSA) in PBS at 37^o C. All reactions were performed in humidity chamber to prevent evaporation.

Biotinylation of secondary antibody. Biotinylation was carried out with Sulfo-NHS-LC-Biotin (sulfosuccinimidyl-6-[biotin-amido]hexanoate) reagent (Pierce). 50 µg of anti-PSA antibody were allowed to react with 0.007 µmol biotinylation reagent in PBS for 2 h at 4^{o} C. The labelled secondary antibody was purified with Zeta Desalt Spin Columns (Pierce) and eluted in 400 µl PBS. Dilution of 1:10 in PBS was used in the electrochemical immunosensor.

Detection. 20 µl samples containing PSA was applied for 1 h to the electrode surface. Then secondary antibody labelled as described, was applied for 1 h. The electrode was washed with 0.1 M Tris-HNO_3, pH=7 containing 0.05% Tween-20. 20 µl streptavidin-alkaline phosphatase (2.5 ng/ml in 0.1 M Tris-HNO_3, pH=7, 2 mM $Mg(NO_3)_2$) were then applied for 30 min. The electrode was washed with buffer as previously, and exposed to 30 µl solution of 0.1 M Tris-HNO_3, pH=9.8, containing 20 mM $Mg(NO_3)_2$, 1 mM 2-phospho-*L*-ascorbic acid and 1 mM $AgNO_3$, for 40 min. Electrode was washed in water and square wave voltammetry was carried out as described to strip the adsorbed silver.