Supplementary Information

Methods

Reagents and Materials

Tetrabutylammonium tetrafluoroborate (NBu_4BF_4), sodium tetrafluoroborate ($NaBF_4$), para-aminobenzoic acid, aniline, 4-mercaptobenzoic acid, 1-propanethiol, ferricyanide ($K_4Fe(CN)_6$), 1,3-Dicyclohexylcarbodiimide (DCC), ferrocenecarboxaldehyde, sodium cyanoborohydride, acetonitrile (CH_3CN, HPLC grade), and absolute ethanol were obtained from Sigma Chem. Co. (Sydney, Australia). Sulfo-NHS-Biotin was purchased from Pierce Chemical Company. Anti-biotin from goat, bovine serum albumin fraction V (BSA), anti-pig IgG, and free biotin were purchased from Sigma. Molecular wire (MW) was synthesized by following the methods from Tour and coworkers.[1]; details will be reported elsewhere.[2] Polyethelyene glycol (PEG) was synthesized according to the method reported.[3] Ferrocenedimethylamine was synthesized using the procedure from Ossola.[4] Reagent grade dipotassium orthophosphate, potassium dihydrogen orthophosphate, potassium chloride, sodium hydroxide, sodium chloride, sodium nitrite, ammonium acetate, sulphuric acid,

[1] [a]Tour, J. M.; Rawlett, A. M.; Kozaki, M.; Yao, Y. X.; Jagessar, R. C.; Dirk, S. M.; Price, D. W.; Reed, M. A.; Zhou, C.; Chen, J.; Wang, W.; Campbell, I. *Chem. Eur. J.* **2001**, *7*, 5118-5134, [b]Kosynkin, D. V.; Tour, J. M. *Org. Lett.* **2001**, *3*, 993-995.

[2] Liu, G. Z.; Gooding, J. J. *Langmuir* **2006**, *22*, 7421-7430.

[3] Bahr, J. L.; Yang, J.; Kosynkin, D. V.; Bronikowski, M. J.; Smalley, R. E.; Tour, J. M. *J. Am. Chem. Soc.* **2001**, *123*, 6536-6542.

[4] Ossola, F.; Tomasin, P.; Benetollo, F.; Foresti, E.; Vigato, P. A. *Inorgan. Chim. Acta* **2003**, *353*, 292-300.

hydrochloric acid, methanol and diethyl ether were purchased from Ajax Chemicals Pty.Ltd. (Sydney, Australia). All reagents were used as received, and aqueous solutions were prepared with purified water (18 MΩ cm^{-1}, Millipore, Sydney, Australia). Phosphate buffered saline (PBS) solutions were 0.15 M NaCl and 0.1 M K_2HPO_4/KH_2PO_4 and adjusted with NaOH or HCl solution to pH 7.3. Phosphate buffer solutions used in this work were 0.05 M KCl and 0.05 M K_2HPO_4/KH_2PO_4 and adjusted with NaOH or HCl solution to pH 7.0.

2.2 Electrochemistry measurements

All electrochemical measurements were performed with a BAS-100B electrochemical analyser (Bioanalytical System Inc., USA) and a conventional three-electrode system. GC electrodes (Bioanalytical Systems Inc., USA) were prepared from 3mm-diameter glassy-carbon rods embedded into epoxy resin and were used as working electrode. Platinum foil and a Ag/AgCl (3.0 M NaCl) electrode were used as the counter and reference electrodes. All potentials reported *versus* the Ag/AgCl reference electrode at room temperature. All cyclic voltammetry (CV) measurements were carried out in pH 7.0 phosphate buffer. The surface coverage of ferrocene was determined electrochemically using a CV from integration of the oxidation or reduction peaks.

2.3 Modification of GC Electrodes with mixed monolayer of MW and PEG

Commercial GC electrodes (Bioanalytical System Inc., USA), 3-mm-diameter rods, were polished successively in 1.0, 0.3, and 0.05 μm alumina slurries made from dry

Buehler alumina and Milli-Q water on microcloth pads (Buehler, Lake Bluff, IL, USA). The electrodes were thoroughly rinsed with Milli-Q water and sonicated in Milli-Q water for 5 min between polishing steps. Before derivatization, the electrodes were dried with an argon gas stream. Surface derivatization of GC electrodes was carried out in an acetonitrile solution of 1 mM aryl diazonium salt and 0.1 M $NaBu_4BF_4$ using CV with a scan rate of 100 mV s^{-1} for two cycles between +1.0 V and -1.2 V. The diazonium salt solution was deaerated with argon for at least 15 min prior to derivatization. The electrodes were rinsed with copious amounts of acetonitrile and then water and dried under a stream of argon prior to the next step.

2.4 Covalent Coupling of Ferrocenedimethylamine on MW and PEG Modified GC Electrodes

Covalent attachment of ferrocenedimethylamine to carboxylic acid terminated monolayers was achieved by dipping the MW and PEG modified GC electrodes into the absolute ethanol solution containing 40 mM 1,3-Dicyclohexylcarbodiimide (DCC) and 5 mM ferrocenemethyldiamine overnight. DCC was used for the activation of terminated carboxylic acid groups of the MW.

2.5 Immobilization of biotin and anti-biotin on ferrocenedimethylamine modified GC electrode surface

After immobilization of ferrocenedimethylamine, the GC substrate covered with amine terminal groups was immersed into a 1 mg mL^{-1} solution of NHS-biotin in PBS (pH 7.3)

for 2 h at 4℃ to attach biotin to the free terminal amines on the surface bound ferrocenedimethylamine. This system was referred to as the sensor. Subsequently, the sensor was rinsed with copious amount of water and PBS followed by immersion into a solution containing 0.5 μM anti-biotin dissolved in PBS for 20 min at 4℃ to complete the derivatization (Scheme 1).

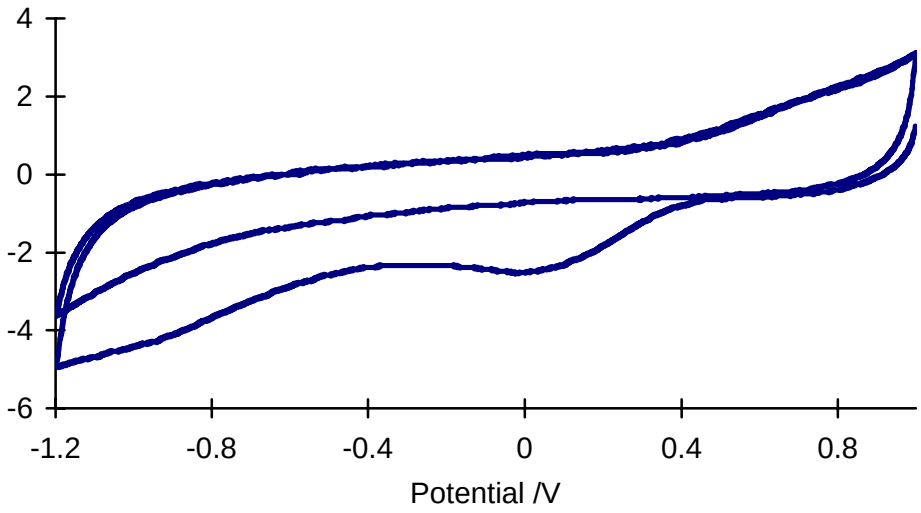

Figure S1: Cyclic voltammograms of a GC electrode in a 1 mM MW and PEG mixed diazonium tetrafluoroborate, acetonitrile/0.1 $MNBu_4BF_4$ solution (molar ratio of MW to PEG is 1:20) at a scan rate of 100 mV s^{-1} for two cycles between +1.0 V and -1.2 V. The peak at 0 V is due to the reductive adsorption of the aryl diazonium salts with the absence of this peak in the second cycle indicating the electrode surface is passivated by the covalently attached layer derived from these diazonium salts.

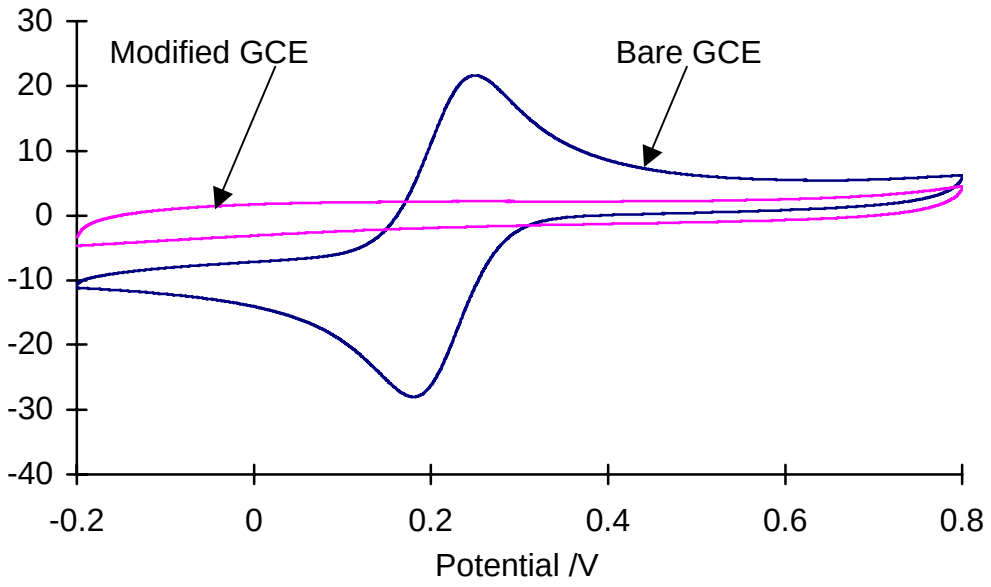

Figure S2: Cyclic voltammograms of the bare and mixed monolayer of MW and PEG modified GC electrodes in ferricyanide solution (1 mM; KCl, 0.05 M; phosphate buffer; pH 7.0) at a scan rate of 100 mV s^{-1} demonstrating the passivation of the electrode by the reductively adsorbed layer.

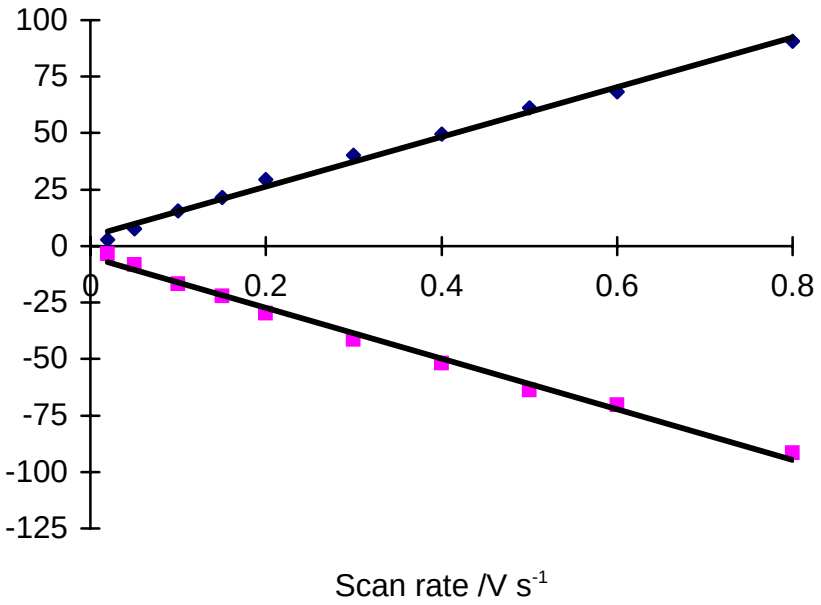

Figure S3: Peak current *versus* the scan rate for the cyclic voltammograms after the

attachment of ferrocenedimethylamine The linearity of park current with scan rate is indicative of a surface bound electrochemical process thus providing evidence the ferrocene is surface bound. Diamonds: anodic scan; Squares: cathodic scan

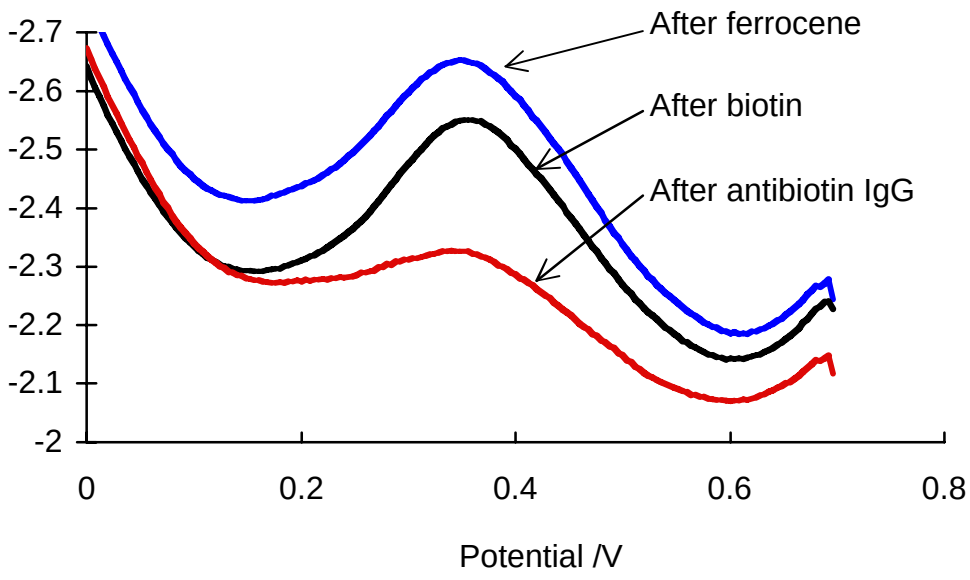

Figure S4 Osteryoung Square Wave Voltammetry (OSWV) of the mixed monolayer of MW and PEG modified GC surfaces after the step-wise attachment of ferrocenedimethylamine, biotin and 0.5 μM anti-biotin in 0.05 M phosphate buffer (0.05 M KCl, pH 7.0) at a scan rate of 100 mV s^{-1}. This is comparable data to that shown in Figure 2a.

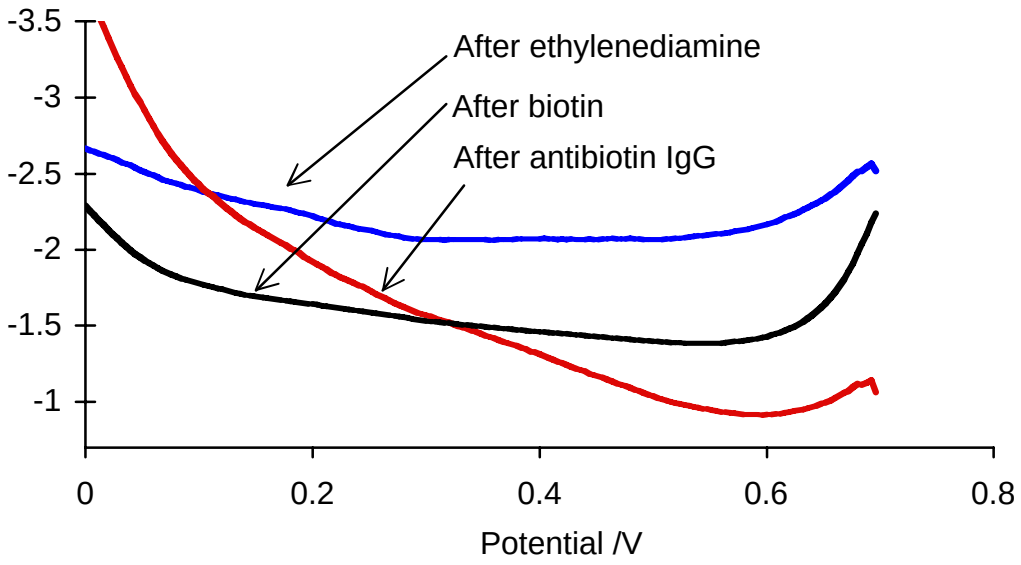

Figure S5: Control data showing that with the attachment of ethylenediamine rather than ferrocene dimethylamine there is no electrochemistry at any stage in the electrode fabrication.

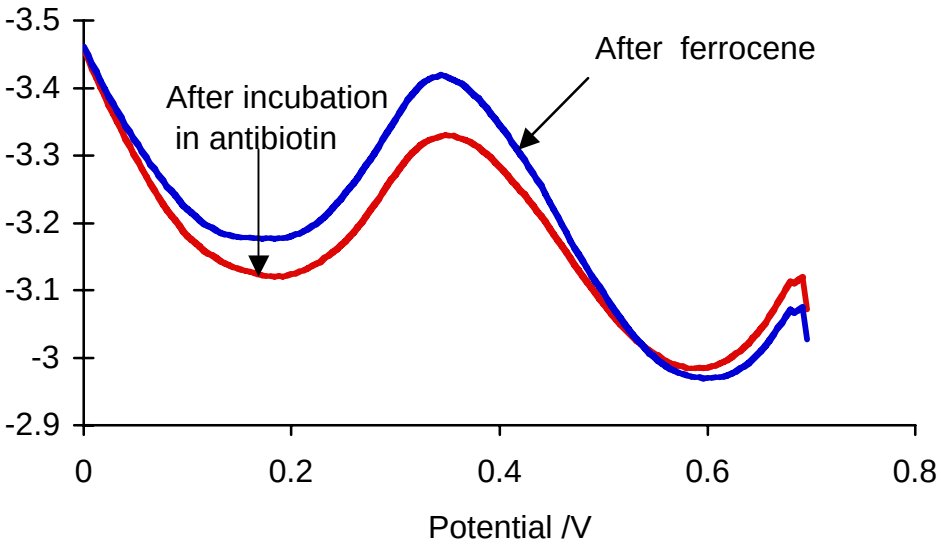

Figure S6: The response of the electrode before and after exposure to 0.5 μM anti-biotin when there is no biotin attached to the end of the ferrocene. The only slight attenuation in current upon exposure of the anti-biotin demonstrates the ability of the interface to resist non-specific binding of the antibody.

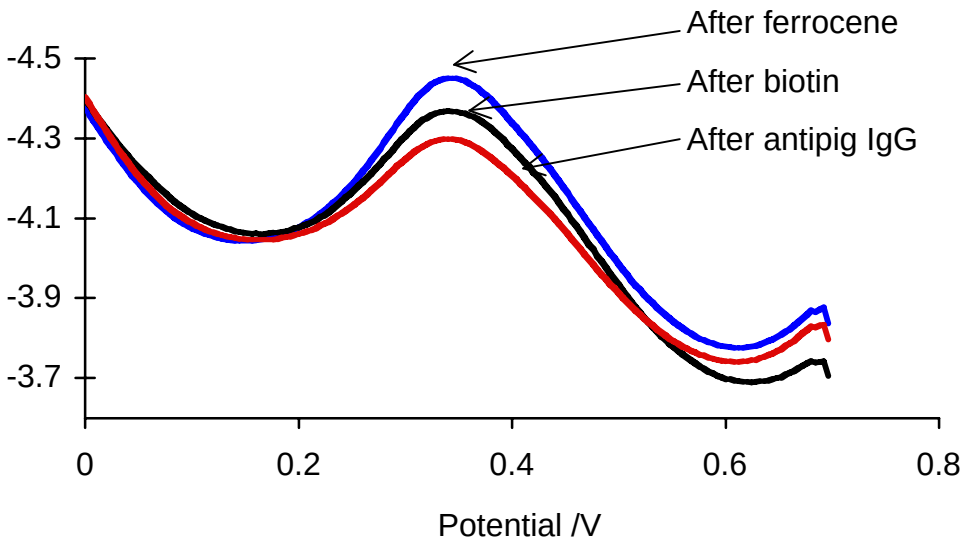

Figure S7: The response of the electrode a) after attachment of ferrocene dimethylamine, b) after attachment of biotin, and c) after exposure to 0.5 μM anti-pig IgG antibodies that are not selective for biotin. The only slight attenuation in current upon exposure of the anti-pig IgG antibodies demonstrates the ability of the interface to resist non-specific binding of the antibody.

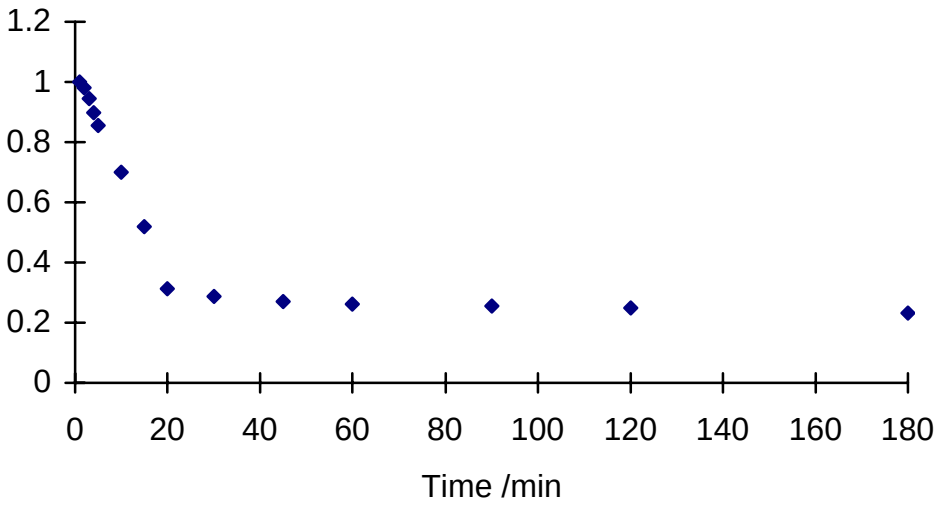

Figure S8 Relative current from the OSWV for the biotin modified GC surfaces after incubation in 0.5 μM anti-biotin for different incubation times. Relative current is obtained by dividing the current before the incubation of anti-biotin with the current after the incubation of anti-biotin.

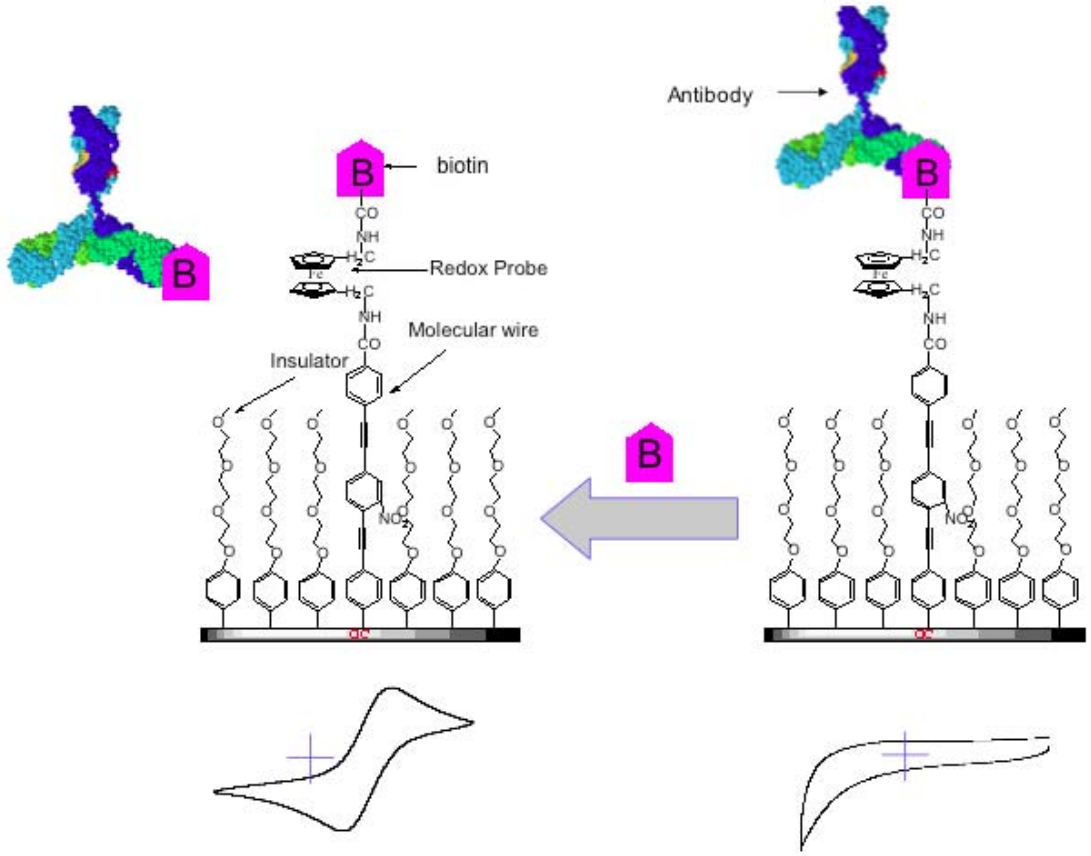

Figure S9: A schematic of the immuno-biosensor for detecting small molecules.

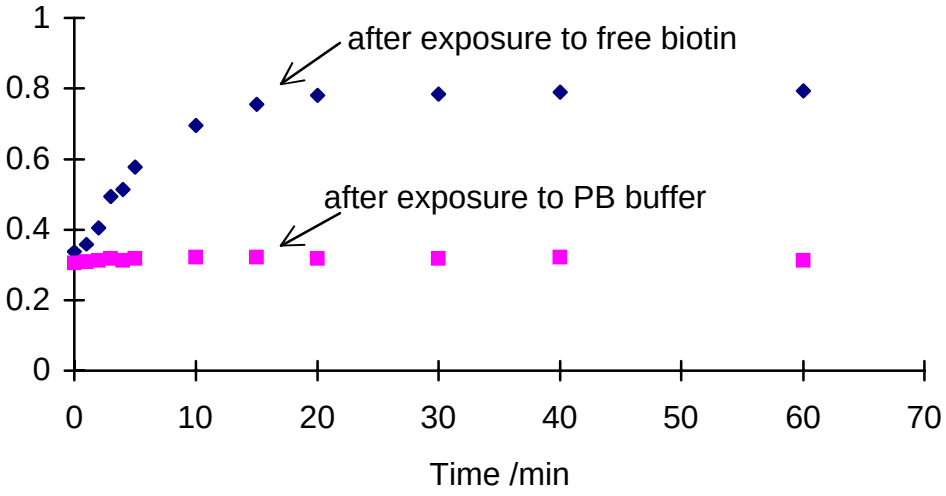

Figure S10: Relative current of anti-biotin modified GC surface after exposure of 0.3 mg mL^{-1} biotin in 0.05 M phosphate buffer (pH 7.0) for different time and after

exposure to phosphate buffer containing no biotin. Relative current is obtained by dividing the current before the exposure of biotin with the current after.