

## Supplementary data

### 2. Experimental Section

#### 2.1. Apparatus and Electrodes

A Rotring T 0.5 pencil was used as a holder for the graphite lead (Tombo HB model 0.5 mm). Electrical contact with the lead was obtained by soldering a metallic wire to the metallic part. The pencil lead was held vertically with 1.5 cm of the lead protruding outside during each measurement (1cm of which was immersed into the solution). In each experiment, the concentrations of the buffers and DNA solutions were optimized. For denaturation of the PCR samples, a Techne-512 Thermal Cycler (United Kingdom) was used.

#### 2.3.1. Procedure for optimization studies

##### DNA immobilization via adsorption method

PGE surfaces were pretreated before each assay by applying a potential of +1.40 V vs. reference electrode for 60 s in 0.50 M acetate buffer solution (pH: 4.8, ABS). Probe was immobilized onto the activated surface of PGE for 30 min via wet-adsorption method. For this purpose, electrodes were immersed into ABS buffer solution containing 7  $\mu\text{g/mL}$  of probe DNA. After immobilization, probe modified PGEs were rinsed with PBS for the removal of the unbound DNA from the electrode surface.

##### Hybridization between probe-covered surface and its synthetic target

The probe-covered graphite electrode was immersed into 12  $\mu\text{g/mL}$  synthetic target or synthetic non-complementary DNA solutions which were prepared using hybridization buffer (0.05 M potassium phosphate buffer solution containing 500 mM NaCl, pH: 7.4) for 30 min using adsorption method. After the hybridization event, electrodes were immersed into the washing buffer (PBS, pH: 7.4) once to remove unbound DNA.

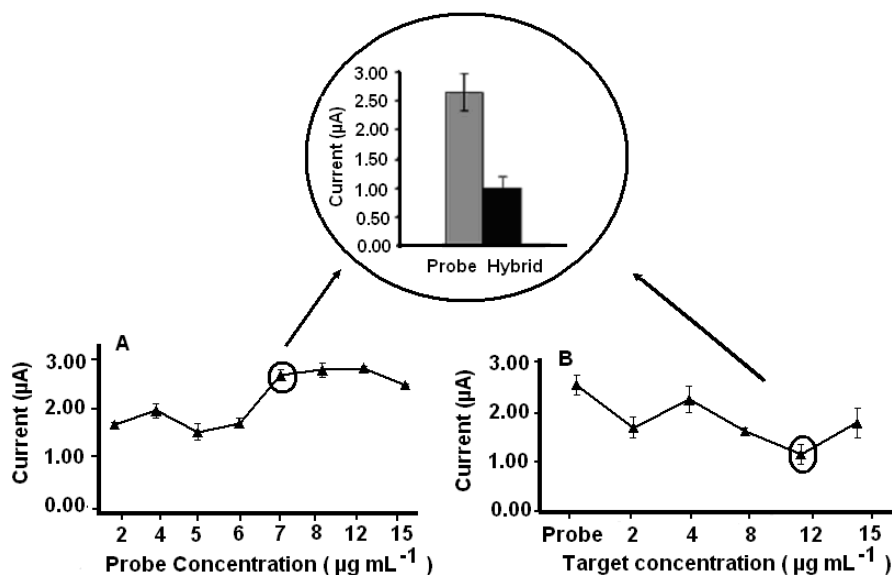
##### Voltammetric transduction of guanine

The oxidation signal of guanine was measured by DPV in blank ABS by scanning from +0.75 to +1.40 V. DPV measurements were performed using 50-mV modulation amplitude, 8-mV step potential, and 15 mV/s scan rate vs. the Ag/AgCl reference electrode. The raw data were treated using the Savitzky-Golay filter (level 2) of the General Purpose Electrochemical Software of Eco Chemie by moving average baseline correction with a “peak width” of 0.01 V. The peak height was evaluated by the GPES software.

### 3. Results and Discussion

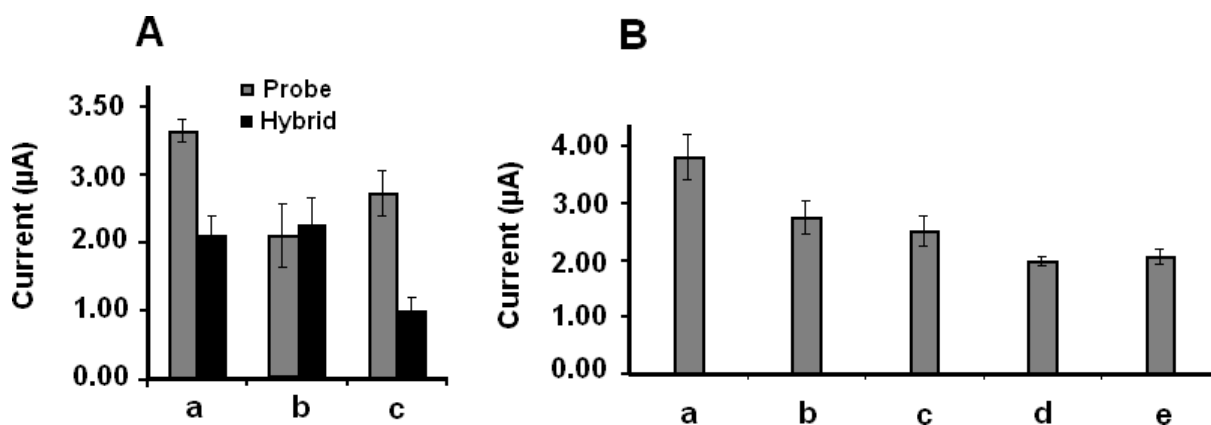
#### 3.1. Optimization of the hybridization conditions between synthetic oligonucleotides based on guanine signal

The effects of the probe concentration on the guanine signals were evaluated according to the Figure S1A. The probe concentration was changed from 2 to 15  $\mu\text{g mL}^{-1}$  and it was observed that increasing the probe concentrations led to an increase of the guanine response up to 7  $\mu\text{g mL}^{-1}$  and it was almost stable between 7 and 15  $\mu\text{g mL}^{-1}$ . The concentration of 7  $\mu\text{g mL}^{-1}$  was also used for AuNPs-modified electrode and we were evaluated it enough amount for the immobilization of probe. Figure 1B shows the effects of target concentration using synthetic oligonucleotides on hybridization reaction. Probe concentrations were kept constant at 7  $\mu\text{g mL}^{-1}$ , while the concentration of the complementary target was increased up to 15  $\mu\text{g mL}^{-1}$ . Thus, increasing the target concentrations, hybridization occurred in a different ratios as seen in Figure S1B by reduction of the guanine signal in hybrid<sup>29</sup>. The best differentiation of guanine signals between probe and hybrid were obtained at a target concentration of 12  $\mu\text{g mL}^{-1}$ . So, this value was chosen as optimum target concentration for further experiments.



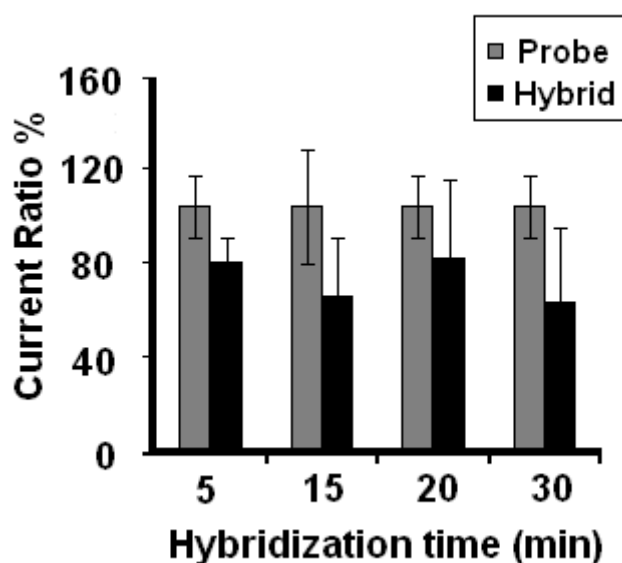
**Figure S1.** (A) Effect of probe concentration on the surface coverage of transducer and (B) the effect of target concentration upon the hybridization event using DPV response of the guanine oxidation signal at different concentrations. The inset of Figure S1 presented optimum concentrations of probe and target for hybridization detection.

Figure S2A shows the influence of pH and composition of probe immobilization buffer on the hybridization reaction with the use of optimum concentrations of synthetic probe and target. According to results, since maximum discrimination rate between probe and hybrid responses was obtained with ABS (pH: 4.7), it was chosen as the immobilization buffer. The effect of ionic strength on hybridization was shown in Figure S2B. According to the relationship between the salt concentration of target solution and hybridization, the optimum ionic strength was chosen as 0.5 M NaCl for further experiments to monitor effective hybridization response of probe and its complementary target. The better and more reproducible results were obtained from PCR samples based on the MDB signal using 5X SSC buffer (Figure 3 and 4).



**Figure S2:** (A); The effect of immobilization buffer (pH and composition) on the hybridization reaction: (a) 5X SSC (pH: 7.40), (b) 0.02 M PBS (pH: 7.40), (c) 0.5 M ABS (pH: 4.80). Hybridization buffer is 0.02 M PBS (pH: 7.40) with 20 mM NaCl; gray columns represent the guanine response of only probe-DNA modified surface and black columns show the response of hybridization event between the probe and target. (B); The effect of ionic strength on hybridization; (a) only probe signal before hybridization with its target, NaCl concentrations of hybridization buffer are (b); 0.05 M, (c); 0.1 M, (d); 0.5 M, and (e); 1 M. The 7 µg mL<sup>-1</sup> concentration level of probe DNA immobilization on PGE for 30 min; hybridization with 12 µg mL<sup>-1</sup> target sequence on CGE surface by adsorption in the vial for 30 min; washing electrodes with washing buffer (PBS, pH: 7.4) for once and measurement of the oxidation signal of guanine using DPV in blank ABS with 20 mmol/L NaCl by scanning from +0.75 to +1.40 V. DPV measurements, 50-mV modulation amplitude, 8-mV step potential, and 15 mV/s scan rate vs. the Ag/AgCl reference electrode.

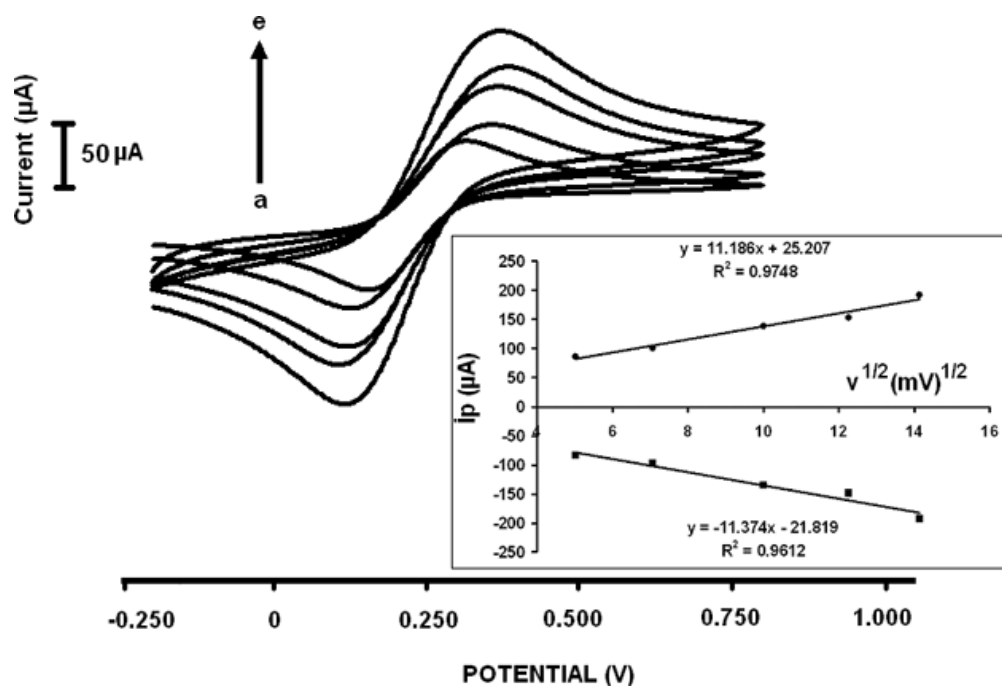
The effect of the time of hybridization (Fig. S3) on the detection of the Influenza B virus region were also tested with synthetic oligonucleotides. The hybridization time was changed from 5 to 30 min and the results were obtained by the oxidation signal of guanine which were normalised as current ratio (%). The best detection of hybridization was observed using 30 min hybridization time. Therefore, optimal time condition for hybridization of the sensor were chosen as 30 min.



**Figure S3:** The effect of hybridization time on guanine oxidation response on: (gray columns); only probe modified transducer, (black columns); hybridization signals obtained from hybrid which are occurred between probe and target sequences. Hybridization time was changed from 5 to 30 min. Other conditions are as in Figure- S2.

The effect of scan rate (from 25 to 200 mV/s) on the oxidation and reduction peaks of  $\text{Fe}(\text{CN})_6^{3-/4-}$  was investigated to obtain an information about electrochemical behavior of AuNPs-modified sensor surface (Fig S4). The anodic and cathodic peak currents of  $10^{-2}$  M  $\text{Fe}(\text{CN})_6^{3-/4-}$  were evaluated and the results showed that the process is diffusion controlled.

In this study it is aimed to show that the behaviour of carbon transducer with adsorbed gold nanoparticles. Eventually, it was found that electrochemical events which realized onto the electrode is diffusion controlled.



**Figure S4:** Cyclic voltammograms of  $10^{-2} \text{M Fe(CN)}_6^{3-/4-}$  at AuNPs modified sensor surface at different scan rates: (a) 25; (b) 50; (c) 100; (d) 150; (e) 200  $\text{mV s}^{-1}$ . The inset of figure is the plot for dependence of peak heights on square root of scan rate.