Supplementary material for

Miniaturized electrochemical platform with integrated PDMS reservoir for label-free DNA hybridization detection using nanostructured Au electrodes

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Annexure 1 The electrochemical experimental setup



Figure 1. Pictorial representation of the experimental arrangement showing a biosensor interfaced with the CH66oE electrochemical workstation using the micro USB terminal. The enlarged view of a device connected to a male USB connector is also shown.

Annexure 2 Raman spectroscopic analysis of electrodeposited Au nanostructures :



Figure 2. Raman spectra of electrodeposited Au nanostructures, showing characteristic peaks for Au metal.

Annexure 3

1. Electrochemical deposition of Au on Ti :



Figure 3 (a). Cyclic voltammogram of bare Ti electrode in 5 mM HAuCl₄ (in 0.1 M Na₂SO₄) solution, showing reduction of Au^{3+} to Au around -0.9 V.



Figure 3 (b). Current-time behaviour for electrochemical deposition of Au on Ti, at -1 V, for 60 seconds.

2. Effect of Au deposition on the working electrode's response to the redox activity of Fe²⁺/Fe³⁺:



Figure 4. Cyclic voltammograms of bare (Ti) and Au electrodeposited (Ti/Au) working electrodes, in phosphate buffered saline, containing 5 mM $[Fe(CN)_6]^{3^{-/4^-}}$ redox couple. As can be seen, for the bare Ti electrode, no redox activity is observed. On the other hand, post Au deposition, clear peaks are recorded for both oxidation and reduction processes at anode and cathode, respectively. The voltammograms are sigmoidal in nature, for reasons explained in the main manuscript.

3. Effect of electrodeposition time on the electrochemical performance of the Au nanostructured working electrodes



Figure 5. Cyclic voltammograms of bare working electrodes, before and after Au electrodeposition at -1 V, with different electrodeposition time.

Note, with increasing the electrodeposition time from 30 to 60 sec, the overall system response increases, which can be interpreted from the increasing peak current. This can be attributed to the enhancement of effective electrode surface area, which gets enhanced as a result of the finer nanostructure formation and area coverage. However, as observed, with further increase in the deposition time, the system response is degraded on the micro electrode. This can be a result of the amalgamation of Au particles on the electrode, which forms micro sized clusters and thereby reduces the effective surface area. To verify these claims, using the Randles-Servik equation, we have estimated the effective surface areas of the electrode for different deposition times. As found, the surface area of the working electrode for the electrodeposition conditions described above, are given as 0.281 cm² (for 30 sec deposition), 0.56 cm² (for 45 sec deposition), 0.768 cm² (for 60 sec deposition), 0.662 cm² (for 90 sec deposition) and 0.436 cm² (for 120 sec deposition).

Note, for these experiments, in order to maintain uniformity, the electrodeposition was carried out from fresh stock solution of equal molarity. Also, in all these cases, the working electrode area is constant (dia: 2 mm), and the voltammograms were recorded in the same electrolyte. Therefore, the variation in the electrode response indeed is a representative of the effect of the morphology of the gold film, which in turn is a consequence of the deposition time.

Annexure 4 Peak current variation with square root of the scan rate:



Figure 6. Variation of cathodic and anodic peak current with square root of the scan rate, for the cyclic voltammograms presented in Fig. 6(a) of the main manuscript.

Annexure 5 Electrode Stability



Stability of the bare working electrodes in the working buffer

Figure 7. Stability of the bare working electrode in the test solution : Cyclic voltammograms at a scan rate of 80 mV/s before and after 30 minute incubation in working buffer. As can be seen, the cyclic voltammograms of the working Au nanostructured electrode do not show any significant alteration after the incubation in the working buffer (PBS with the redox couple). This is an indication of the electrode's stability in the test solution.

Stability of the bioelectrodes



Figure 8. Bar diagram showing the variation in peak current (mean value) of probe modified working electrodes over a period of 4 weeks. The electrodes were stored under refrigeration for the entire storage duration, and their response in terms of DPV measurements were recorded periodically after every week. The error bars associated with the measurement represent the standard deviation calculated using three identical devices.

Annexure 6 Immobilization of probe DNA on Ti/Au working electrode :



Figure 9. DPVs of Ti/Au working electrode before and after probe DNA immobilization. The degradation in the peak current response is an indication of successful immobilization of the DNA probes. The negatively charged probe nucleotides degrade the reaction kinetics at the electrode/electrolyte interface by increasing the overall electrostatic barrier seen by the electrons.

Annexure 7 Reproducibility of the sensor



Figure 10. Bar diagram showing the variation in system response for 5 working electrodes, with a probe and target concentration of 1 μ M. The standard deviation and relative standard deviation values for the same are given by 0.042 and 7.12% respectively.

Annexure 8 Interfacing with Arduino



Figure 11. A Schematic representation of the device interfacing and data acquisition system.



Figure 12. Picture showing the experimental setup for device interfacing and data acquisition.



Figure 13. Amperometric response of probe DNA (concentration: 1μ M) modified working electrodes, with and without any target DNA (concentration: 10μ M), recorded at a time interval of 1 sec.