Supplementary Information

Functional Graphene- Gold Nanoparticles Hybrid System for Enhanced Electrochemical Biosensing of Free Cholesterol

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1. Experimental Procedure

Preparation of Graphene Oxide (GO). Following method proposed by Marcano et al, briefly, a 9:1 combination of concentrated H$_2$SO$_4$/$H_3$PO$_4$ was added to 1 g of graphite flakes and 6 g of KMnO$_4$. At a temperature of 50° C, the mixture was stirred for 12 h. The mixture was cooled to room temperature and was subsequently quenched by adding ~130 mL of ice with 1 mL of 30% H$_2$O$_2$. This mixture was then sifted, filtered and was washed with distilled water, 30% HCl and ethanol. The material having neutral pH was obtained and dried.

Preparation of RGO. Chemical conversion of GO to RGO is obtained using the method proposed by Dan Li et al. Briefly, a 500 mL (0.25 mg mL$^{-1}$) GO dispersion in double distilled water was kept for ultra-sonication for 30 minutes to obtain a light yellowish homogeneous solution. 2 mL of ammonia solution (25%) was added to the above GO solution to achieve a pH≈10. Further, 350 µL of hydrazine hydrate solution ($H_6N_2O$) was added and the solution was kept under ultra-sonication at a temperature of 80°C for two hours. The sonicated solution was stirred at 95°C for 8 h giving the solution a blacker shade of color. The solution is then filtered, washed and dried at 80° C.

Preparation of Gold Nanoparticles (Au NPs) and the Functionalization. Gold nanoparticles were prepared by the trisodium citrate reduction of gold precursor$^{34}$. Briefly 50 mL of HAuCl$_4$ (1 mM) solution was heated till the boiling temperature was reached. While boiling, 5 mL of Trisodium citrate (38.8 mM) solution was added to it. The boiling process was continued for few minutes,
resulting in a darker solution which subsequently changes to wine red, indicating the formation of Au NPs. For the functionalization, 10 mL of Au NPs after centrifugation were re-suspended in 10 mL of DW as the first step. This Au NPs solution was then treated with 1 mL of MUDA (C₁₁H₂₂O₂S, 20 mM) in ethanol and subsequently 5 mL of DW was added to it. This combination was sonicated at 50°C for an hour and kept undisturbed for one day to obtain Fn Au NPs.

**Preparation of RGO- Fn Au NPs Hybrid system.** For the preparation of RGO- Fn Au NPs hybrid system, 50 mg of RGO was well dispersed in 50 mL of DW by sonication. In this RGO dispersion 5 mL of Fn Au NPs colloid was added and sonicated further for 20 minutes at 70°C. Subsequently it was stirred for 1 h in order to have a well distributed RGO-Fn Au NPs hybrid solution. Finally, this solution was filtered and dried at 85°C to get the required hybrid system.

2. UV-Vis Absorption Spectrum of RGO and Au NPs

The UV-vis absorption spectrum of Au NPs and RGO is shown in Figure S1. The Au NPs shows the absorption peak around 525 nm due to surface plasmon resonance band. The RGO has the characteristic absorption peak ~266 nm which arises from the deoxygenation of the GO under the reduction processes.

![Figure S1. UV-Vis absorption spectra of (a) Au NPs and (b) RGO dispersion](image)

3. FTIR Characterization of RGO and RGO-Fn-AuNPs/Enzyme

The FTIR spectra of RGO and RGO-Fn-Au NPs/ITO -Enzymes are presented in Fig. S2. Fig. S2 (a) denotes the FTIR spectra of RGO, showing different functional groups such as C-O, OH and COOH
attached to the basal planes and the edges of RGO. The band ~ 1720 cm\(^{-1}\) represents the stretching vibration of C=O bonds of the carboxylic acid and the prominent band ~1632 cm\(^{-1}\) is attributed to scissor type O-H bending vibration of molecular H\(_2\)O. The FTIR band around 1105 cm\(^{-1}\) of RGO corresponds to the C–O stretching vibration of the alkoxide group. The band around 1660 cm\(^{-1}\) {Fig. S2 (b)} indicates the formation of amide bond in the hybrid system. The N-H stretch ~ 3450 also can be visible in the same spectrum. The broad band extending from 2800 to 3800 cm\(^{-1}\) is owing to the vibration resulting from O–H stretching in different species. The C-H stretching mode of the carboxylic group bands~2900 cm\(^{-1}\) is prominent in Fig S2 (b).

![FTIR Spectra of (a) RGO (b) RGO-Fn Au NPs/ITO](image)

**Figure S2.** FTIR Spectra of (a) RGO (b) RGO-Fn Au NPs/ITO

4. **XRD Pattern of RGO-Fn Au NPs**

The theoretical calculations of electron density distribution on the plane of the pristine graphene, pRGO, pRGO+Au were made in order to see the effect of Au nanoparticles present on the surface. In fig. S4(a), (b) and (c) the actual electron density maps at the plane of graphene, RGO and RGO+Au are shown. Further, to show the electron density change in RGO and RGO+Au over pristine graphene, the electron density difference map were calculated by using program ChemCraft (shown in figure.S4). The difference map shown in fig. S4(d) shows increase in electron density (shown by blue colour) at the carbon atoms at the edge in going from pristine graphene to RGO. The presence of Au atom at the surface shows a significant decrease in electron density (shown by red colour) over the whole carbon atom plane. The decrease in electron density is larger in going from RGO+Au to RGO [fig. S4(f)] compared to the difference between RGO+Au and pristine graphene [fig. S4(e)]. This study shows that the presence of Au at the surface makes the graphene plane electropositive in nature, empowering to get easily attracted towards negative potential. This theoretical investigation supports coherently the results obtained in the CV measurements and in EIS. In the CV measurement the two electrode one ITO coated glass connected to the negative terminal and the other electrode made up of Pt is connected to the positive terminal of the DC voltage source. When the RGO+Au composite is dispersed in electrolyte it can be easily attracted towards the ITO electrode thereby efficiently depositing on it when compared to the RGO and pristine graphene. This efficient deposition of RGO+Au composite on ITO is one of the primary requirements for the fabrication of a
good substrate for the sensing studies. Further, the presence of Au also provides the efficient electron transfer during the CV measurement which further enhances the sensing studies.

**Figure S4.** Electron density distribution studies (Blue denotes the electron rich density region, Green denotes the neutral region and Red denotes the electron deficient region) of (a) pristine graphene (b) RGO, (c) RGO+Au and the differences (d) RGO – pristine graphene, (e) (RGO+Au) – pristine graphene (f) RGO+Au – RGO
6. Specificity, Stability and Reproducibility Studies

**Figure S5.** (a) Specificity plot describing the CV response of RGO-Fn Au NPs/ITO bioelectrode in presence of cholesterol, glucose, ascorbic acid and urea; (b) Stability studies of RGO-Fn Au NPs/ITO bioelectrode measured at a regular interval one week; (c) Reproducibility test conducted for the different RGO-Fn Au NPs/ITO bioelectrodes fabricated through the same experimental condition.