Supplementary Data

Response Studies of ChOx/4-ATP/Au and ChEt-ChOx/4-ATP/Au bioelectrodes by UV-visible Spectrophotometer

UV-visible experiments were carried out for free and total cholesterol sensing and to determine the thermal stability and shelf-life of ChOx/4-ATP/Au and ChEt-ChOx/4-ATP/Au bioelectrodes. The bioelectrodes (0.5x1.0 cm^2) were dipped in 3 ml PBS solution containing 20 µl of HRP (1 mg/ml), 20 µl of o-dianisidine dye (1%), and 100 µl of analyte (cholesterol or cholesteryl oleate solution) and were kept for about 2 min. The variation in the absorbance value was measured at 500 nm after 2 min of incubation of the respective bioelectrodes.

The biochemical reaction occurring at ChOx/4-ATP/Au bioelectrode in the photometric studies can be expressed as:

$$\text{Cholesterol} + O_2 \xrightarrow{\text{ChOx /4-ATP/Au}} \text{4-Cholesten-3-one} + H_2O_2$$

$$H_2O_2 + \underset{\text{(colorless)}}{\text{o-dianisidine (reduced)}} \xrightarrow{\text{(HRP)}} 2H_2O + \underset{\text{(orange color)}}{\text{o-dianisidine (oxidized)}}$$

The biochemical reaction occurring at ChEt-ChOx/4-ATP/Au bioelectrode can be expressed as:

$$\text{Cholesteryl oleate} + H_2O \xrightarrow{\text{ChEt-ChOx /4-ATP/Au}} \text{Cholesterol} + \text{oleic acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{ChEt-ChOx /4-ATP/Au}} \text{4-Cholesten-3-one} + H_2O_2$$

$$H_2O_2 + \text{o-dianisidine (reduced)} \xrightarrow{\text{(HRP)}} 2H_2O + \text{o-dianisidine (oxidized)}$$

(colorless) (orange color)

Effect of Cholesterol and Cholesteryl oleate Concentration

Figure shows the difference between the initial and final absorbance value obtained at a wavelength 500 nm (due to oxidation of *o*-dianisidine) after 2 min of incubation of ChOx/4-ATP/Au in above prepared solution containing different concentrations of cholesterol. It can be seen (Fig. 1S) that the value of absorbance increases linearly with increasing cholesterol concentration in the range of 12.5 mg/dl to 500 mg/dl. Similar experiments have been carried out with ChEt-ChOx/4-ATP/Au with different concentrations of cholesteryl oleate that show detection of total cholesterol upto 400 mg/dl.

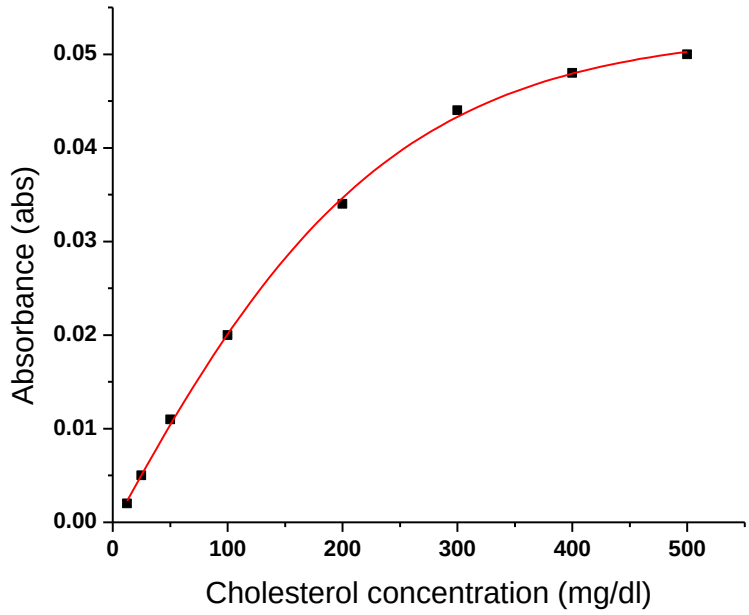

Fig. 1S. Plot of absorbance as a function of cholesterol concentration

Thermal stability of ChOx/4-ATP/Au and ChEt-ChOx/4-ATP/Au bioelectrodes

Fig. 2S (a) & (b) shows variation of observed difference between the initial and final absorbance value measured at 500 nm after 2 min of incubation of ChOx/4-ATP/Au and ChEt-ChOx/4-ATP/Au bioelectrodes at temperatures 15 to 55°C for a fixed analyte concentration of 200 mg/dl . It was observed that the value of absorbance increases with increasing temperature up to ~50 °C, whereafter it shows a decrease, indicating the denaturation of enzyme at temperatures >50 °C. The obtained results show that 4-ATP SAM based cholesterol sensor is extremely stable at temperatures more than normal body temperature (37^{o}C).

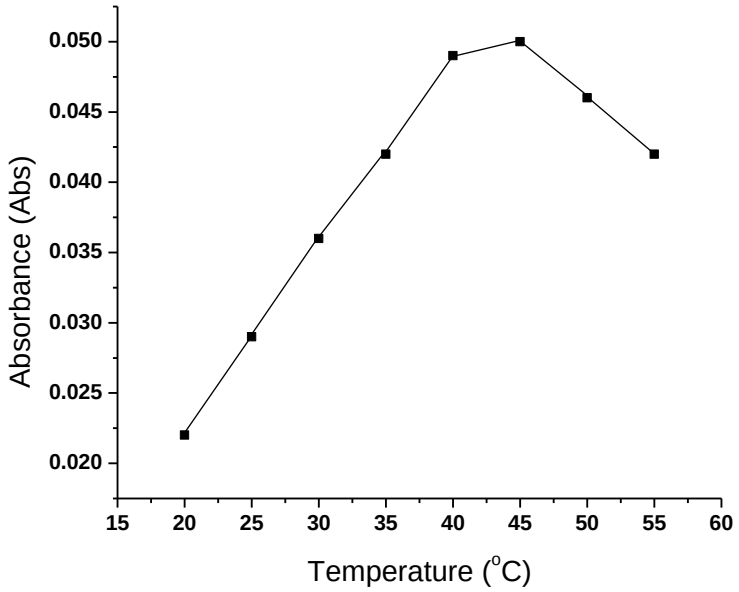

Fig. 2S. (a). Photometric response of ChOx/4-ATP/Au bioelectrode as a function of temperature

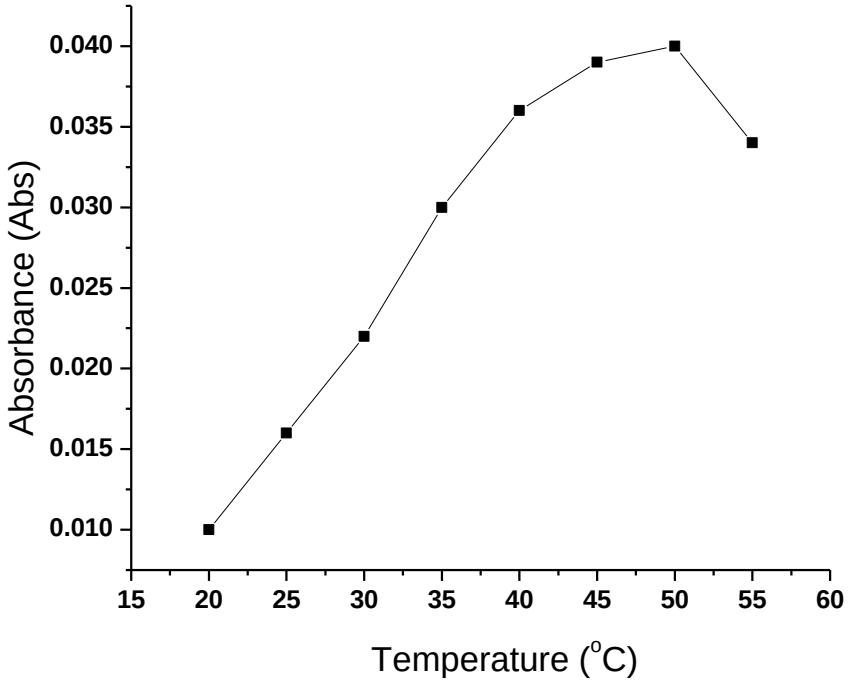

Fig. 2S. (b). Photometric response of ChEt-ChOx/4-ATP/Au bioelectrode as a function of temperature.

Activation energy

The variation of ln (Absorbance) as a function of inverse of temperature for ChOx/4-ATP/Au and ChEt-ChOx/4-ATP/Au bioelectrodes is shown in Fig. 3S (a) and 3S (b). The temperature dependence of absorbance shows an Arrhenius behavior and can be expressed as:

$$A = A_o \exp\left[\frac{-\Delta E}{RT}\right] \qquad \text{(Eq. 4)}$$

where A is the absorbance, R is gas constant, T is temperature (in K), A_o is pre-exponential factor and ΔE is the activation energy (J/mole). Activation energy is defined as the minimum amount of energy required for an enzymatic reaction to occur and its value can be estimated from

the slope of Arrhenius plot. The value of the activation energy for ChOx/4-ATP/Au and ChEt-ChOx/4-ATP/Au bioelectrodes calculated using the slope of Arrhenius plot (Fig. 3S), i.e., plot of ln (absorbance) with inverse of absolute temperature, has been found to be 30.2 kJ/mol and 42.5 kJ/mol, respectively in the lower temperature range.

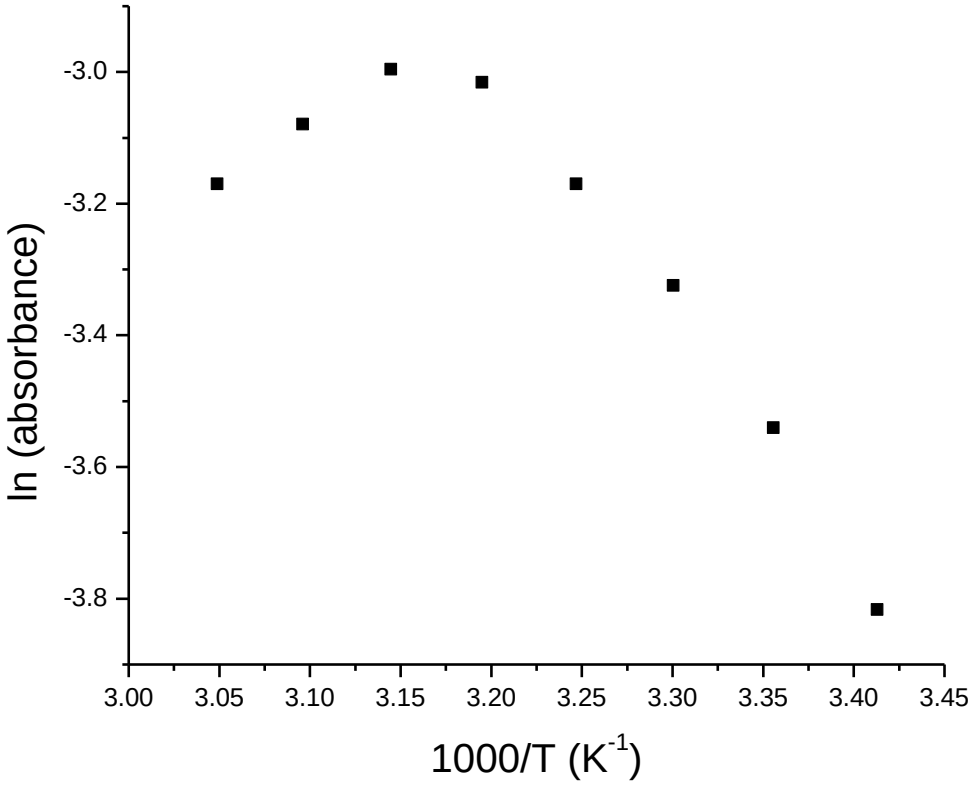

Fig. 3S. (a). Plot of ln (absorbance) vs inverse of temperature for ChOx/4-ATP/Au bioelectrode

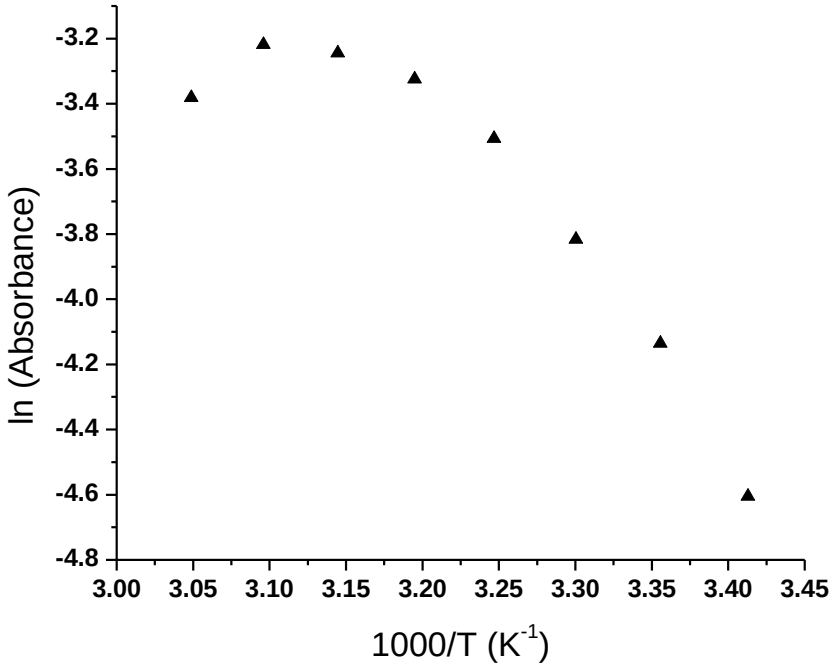

Fig. 3S. (b). Plot of ln (absorbance) vs inverse of temperature for ChEt0ChOx/4-ATP/Au bioelectrode.

Shelf-life studies

UV–visible experiments were also carried out to at regular intervals of a week to investigate the shelf-life of the prepared enzyme electrodes (data not shown). The enzyme electrodes were stored at 4^{o}C when not in use. Activity measurements were done at 30^{o}C with 200 mg/dl concentrations of cholesterol and cholesteryl oleate. The weekly absorbance measurements reveal that ChOx/4-ATP/Au and ChEt-ChOx/4-ATP/Au bioelectrodes are stable for about 4 months with more than 90% of the initial activity.