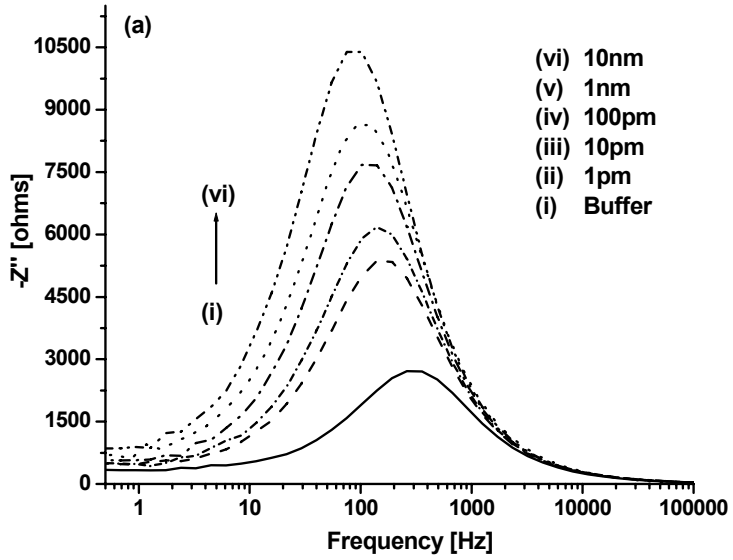
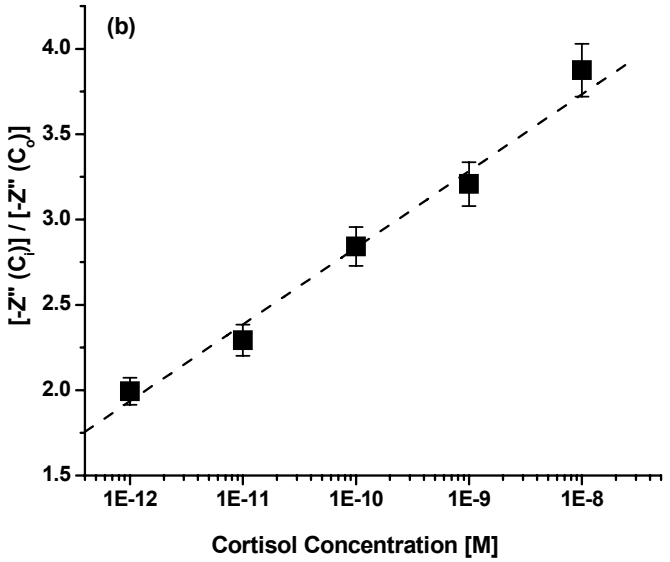
# Supplementary Data

*1. Cortisol response studies of EA-Gly/C-Mab/DTSP/IDμE bio-electrode*

EA-Gly/C-Mab/DTSP/IDμE bio-electrode was utilized to study the interaction between immobilized C-Mab and cortisol concentrations in the range of 1 pM to 10 nM (Supplement Fig.1a). For each concentration, the bio-electrode was incubated in cortisol solution for 30 minutes, followed by PBS washing and EIS spectra recording using PBS (10 mM, pH 7.4) containing 5 mM $Fe(CN)_6^{3-/4-}$ as a redox probe. Supplement fig 1a shows the curve between logarithm of frequency and imaginary part of impedance (-Z"). It is clear that –Z" increases regularly with increasing cortisol concentration.

To compensate for the variation in initial impedance values for individual electrodes, the data was normalized. Normalization was carried out with imaginary impedance data and curve was plotted between [–Z" for desired concentration (–Z" ($C_i$))]/[–Z" of blank EA-Gly/C-Mab/DTSP/IDμE bio-electrode (-Z"($C_o$))] versus logarithm of cortisol concentration (Supplement fig 1b). After normalization, all electrodes with different impedance for EA-Gly/C-Mab/DTSP/IDμE bio-electrode exhibited similar response within the 4% error for each concentration. Normalized data curve in Supplement fig 1b can be characterized using –Z" ($C_i$)/-Z"($C_o$) = 7.33 + 0.449 log $C_{cortisol}$ (M) and reveals linearity for cortisol in the range of 1 pM to 10 nM with the sensitivity of 0.449 $M^{-1}$, correlation coefficient of 0.991 and standard deviation of 0.953. It is clear that after normalization both $R_{ct}$ and –Z" give similar response and can be utilized for sensing.

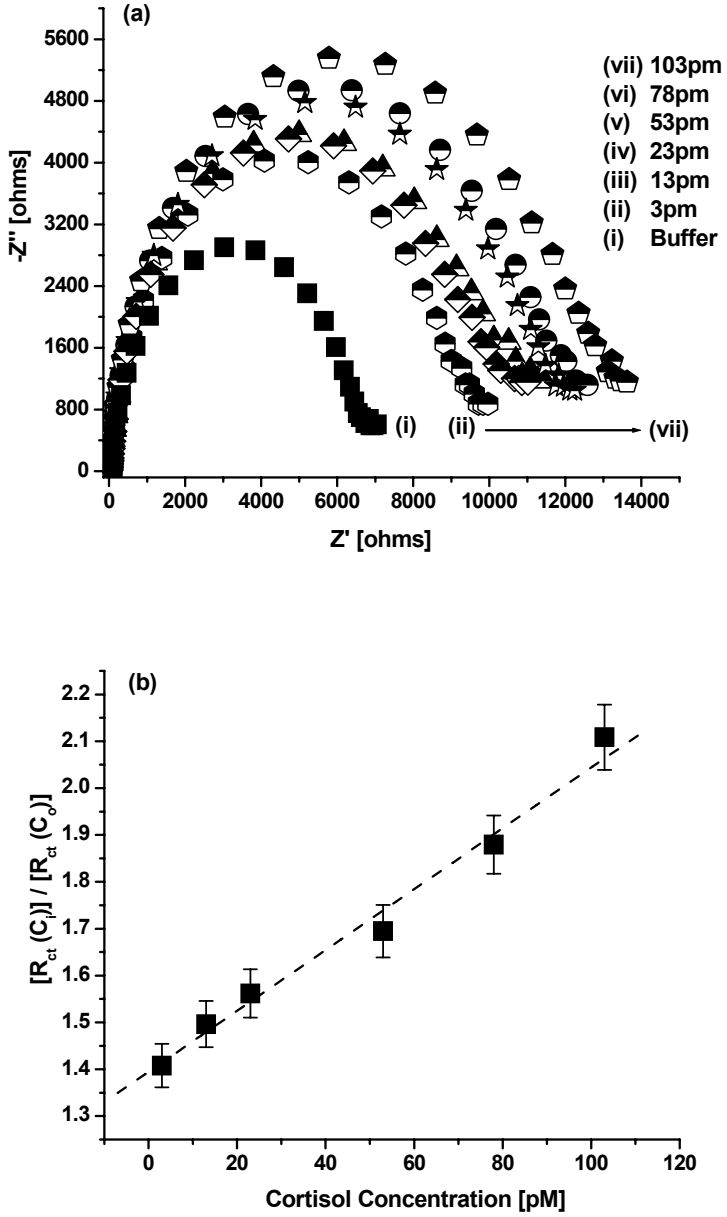





Supplement Fig.1 (a) EIS spectra of EA-Gly/C-Mab/DTSP/IDμE for cortisol concentration (i) buffer, (ii) 1pM, (iii) 10 pM, (iv) 100 pM (v) 1 nM and (vi) 10 nM and (b) normalized data curve for data obtained from EIS studies for different cortisol concentration.

*2. Saliva sample studies*

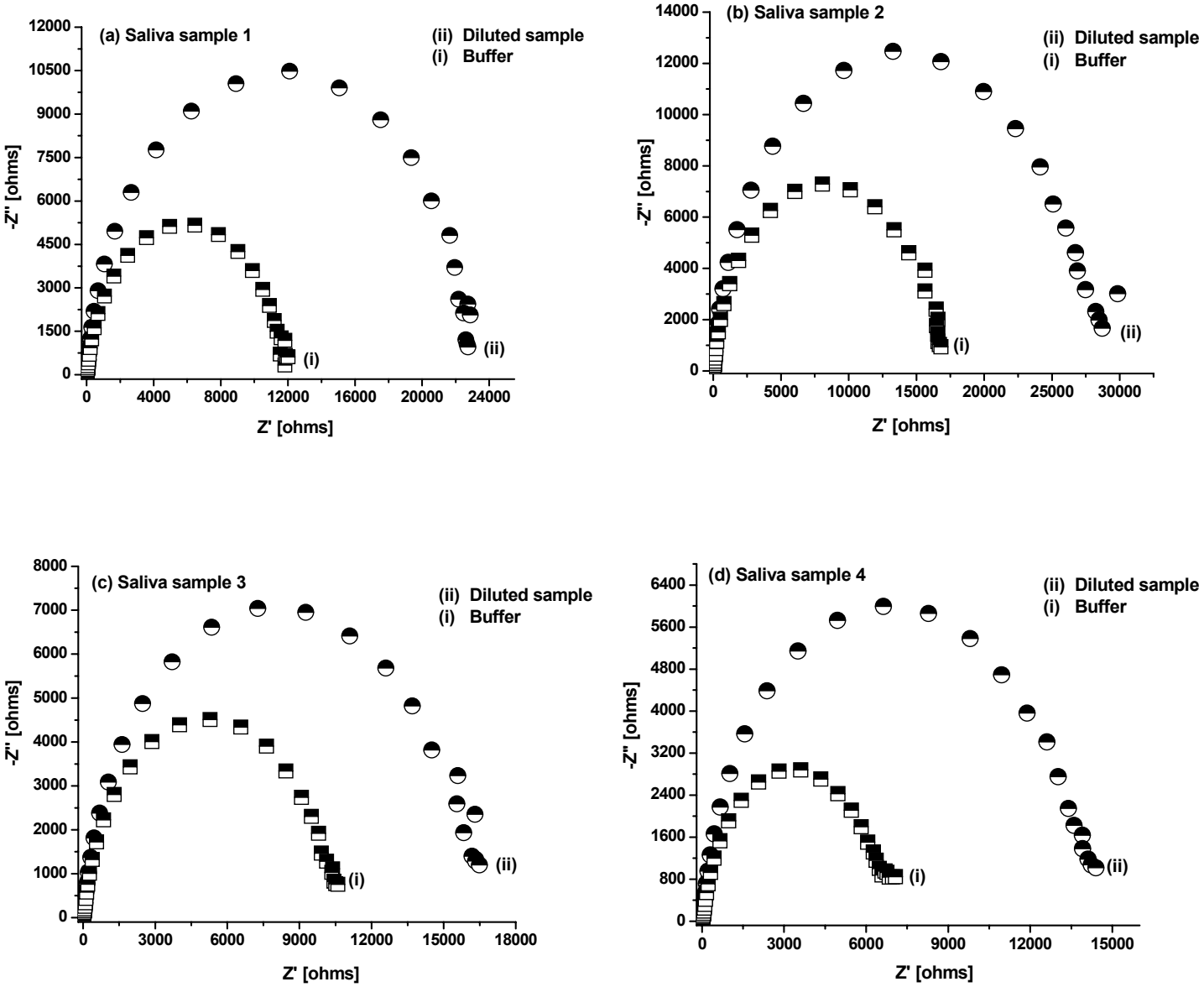
Impedance-based biosensor, described in the present study, was used to analyze the human saliva. Saliva samples of five subjects were analyzed and compared with the commercially available competitive ELISA results obtained for the same samples (Table 1). To test the real sample, they were diluted 100 times in PBS (10 mM, pH 7.4) before analysis. For 1 to 100 pM range a new standard curve with normalization ($R_{ct}$ ($C_i$)/$R_{ct}$ ($C_o$)) was plotted (Supplement figure 2b) by testing various concentration of cortisol in 1000 time diluted saliva diluent (Initial value 3.0 nM) (Supplement figure 2a). For each concentration, the bio-electrode was incubated in the cortisol solution for 30 minutes, followed by PBS washing and EIS spectra recording using PBS (10 mM, pH 7.4) containing 5 mM $Fe(CN)_6^{3-/4-}$ as a redox probe.

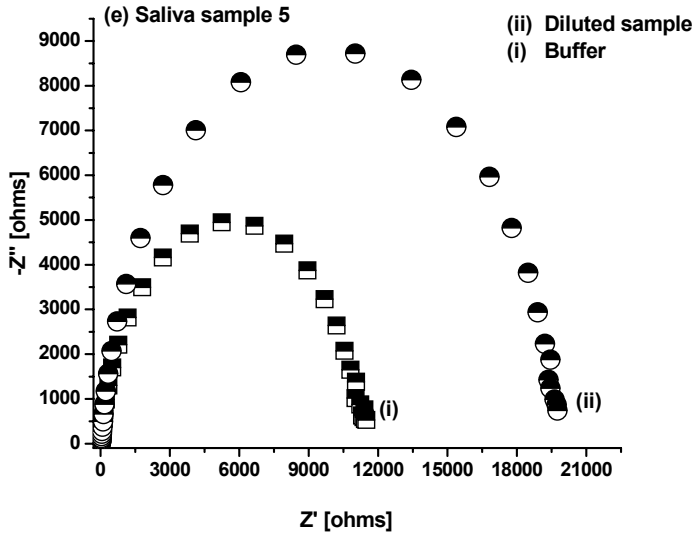



Supplement Fig 2. (a) EIS spectra of EA-Gly/C-Mab/DTSP/IDμE for cortisol concentration in diluted saliva (i) buffer, (ii) 3 pM, (iii) 13 pM, (iv) 23 pM, (v) 53 pM, (vi) 78 pM and (vii) 103 pM. (b) Normalized curve for data obtained from EIS studies for different cortisol concentration.

For each real saliva sample, EIS spectrum was first recorded for fresh EA-Gly/C-Mab/DTSP/IDμE bio-electrode, in PBS buffer to get the base value. Bio-electrode previously analyzed was then incubated with the sample diluted 100 times in PBS buffer

for 30 mins, followed by PBS washings, and EIS spectra recording in PBS (10 mM, pH 7.4) containing 5 mM $Fe(CN)_6^{3-/4-}$ as a redox probe (Supplement Fig 3a to 3e). For each bio-electrode, diameter of the Nyquist plots was found to increase after incubation with saliva sample. After testing, $R_{ct}$ value observed for 100 times diluted sample was normalized and the cortisol level was estimated using the standard curve (supplement fig 2b). Observed values were compared with the values obtained with the commercial ELISA. Both sets of data follow the similar trend which means that the bioelectrode can be utilized for real sample analysis (Table 1).






Supplement Fig 3 (a-e). EIS spectra of EA-Gly/C-Mab/DTSP/IDμE for cortisol in real saliva sample (i) buffer and (ii) 100 time diluted sample.