# Supplemental Information

## Cortisol detection in undiluted human serum using a sensitive electrochemical structure switching aptamer over an antifouling nanocomposite layer

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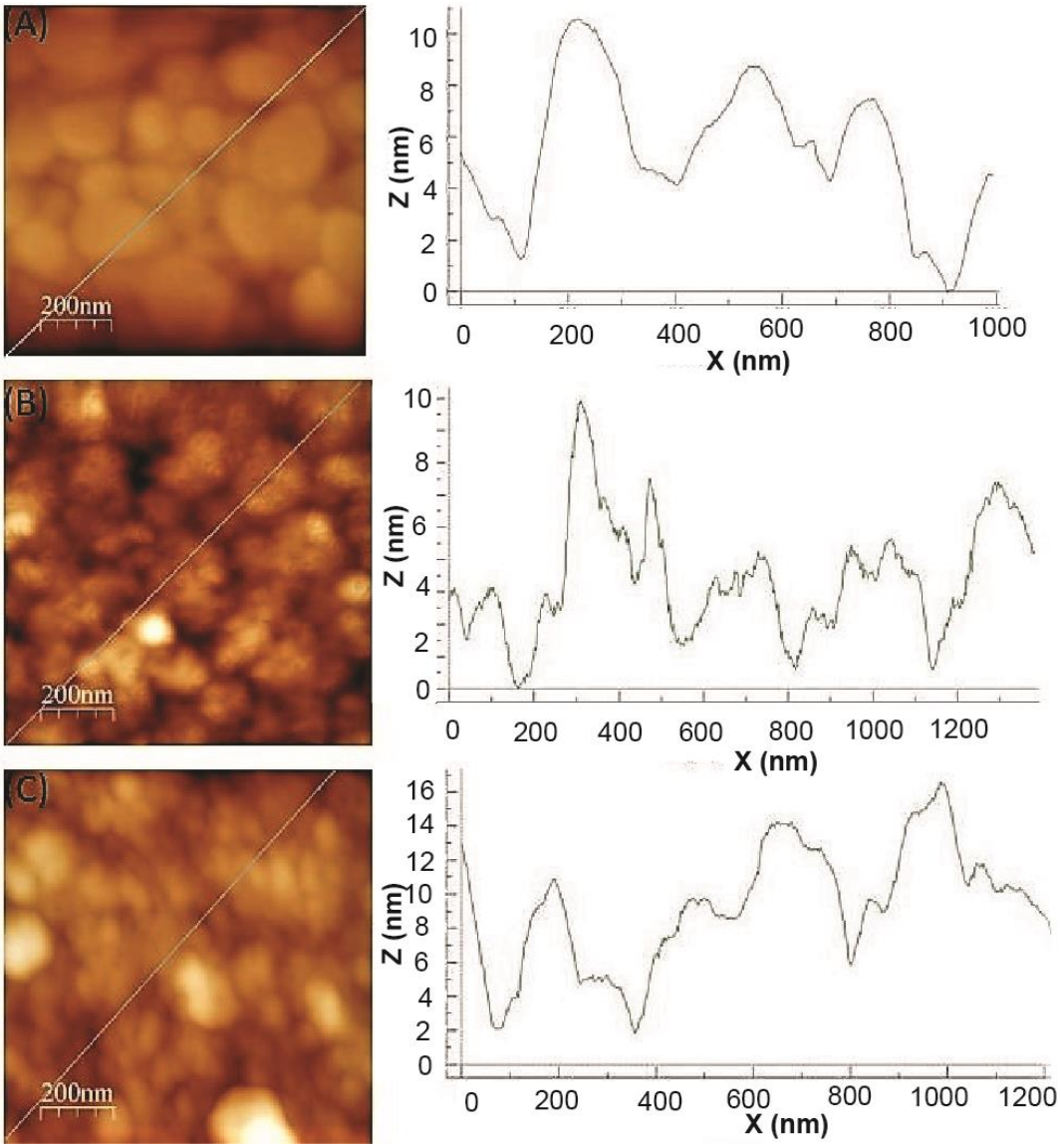


**Figure S1**. **Atomic force microscopy (AFM) measurements.** The surface profile of **(A)** bare gold surface, **(B)** uniform nanocomposite surface, and **(C)** aptamer over the gold electrode. The corresponding surface roughness along the path is also shown.

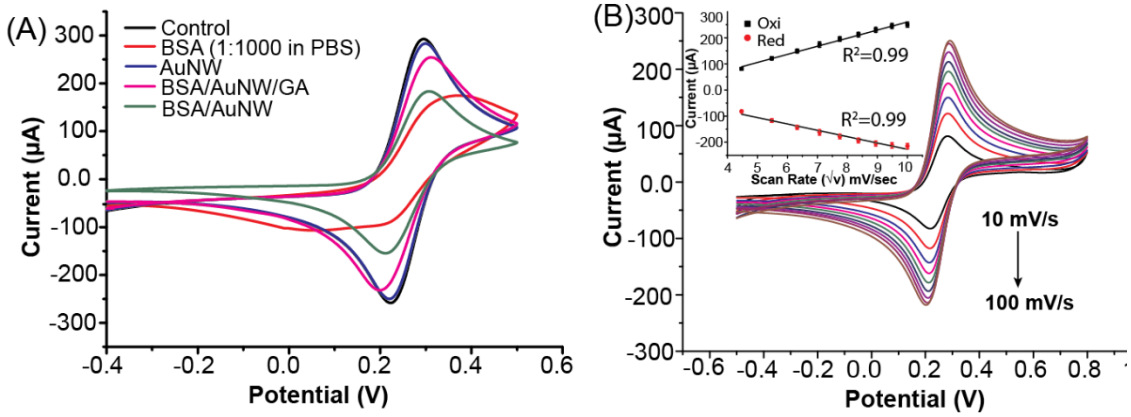


**Figure S2. Sensor characterization studies. (A)** Typical voltammograms showing oxidation and reduction peaks of control (plain Au electrode), AuNW (1:1000 dilution), BSA/AuNW, and BSA/AuNW/GA (nanocomposite). **(B)** Scan rate studies of the electrode. Inset shows extracted redox peak mean current values versus the square root of scan rate.

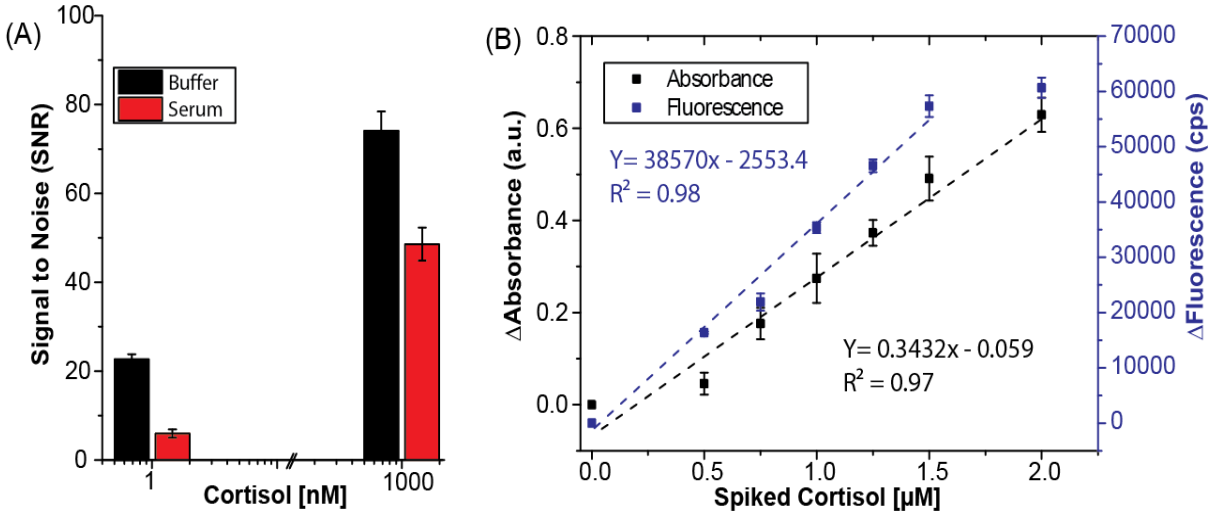


**Figure S3**. **Signal-to-Noise (SNR) ratio and standard spectroscopy studies. (A)** The SNR from lowest to highest cortisol concentration in buffer and undiluted serum. **(B)** Assessment of analytical performance of aptasensor with standard absorbance[1] and fluorescence[2] spectroscopy methods. The ethanolic cortisol spiked in concentrated sulphuric acid at various concentrations and incubated at 37 °C for 10 min before measurement. The fluorescence signal was recorded at emission/excitation of 520/480 nm. The spectroscopy experiment was performed on a Synergy HTX multi-plate reader. The SNR was calculated using the first standard deviation method using the formula (Test current density – background current density/ √ background current density)×100 where spiked cortisol in buffer/saliva was considered the test and unspiked buffer/saliva as background.

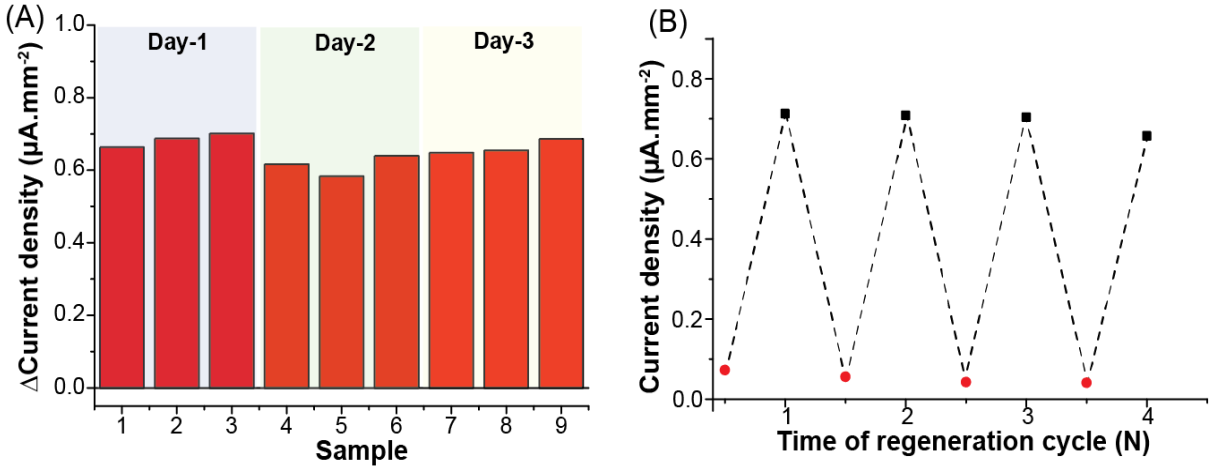


**Figure S4**. **Reproducibility and regeneration assay**. **(A)** Measured voltammograms using DPV with aptasensor with cortisol spiked (1 µM) in buffer on different days. The determined intra- and inter-batch relative standard deviation (RSD) were 3.45% and 5.68%, respectively. The RSD was calculated as RSD (%) = standard deviation (SD)/mean current density×100. **(B)** The aptasensor regeneration with 1 µM of cortisol after the sensor was treated with 1× PBS and 1M NaCl at pH 4.5 for 15 min used for different cycles. The red circles show the preliminary and regenerated signal after being challenged with cortisol shown by black symbols.

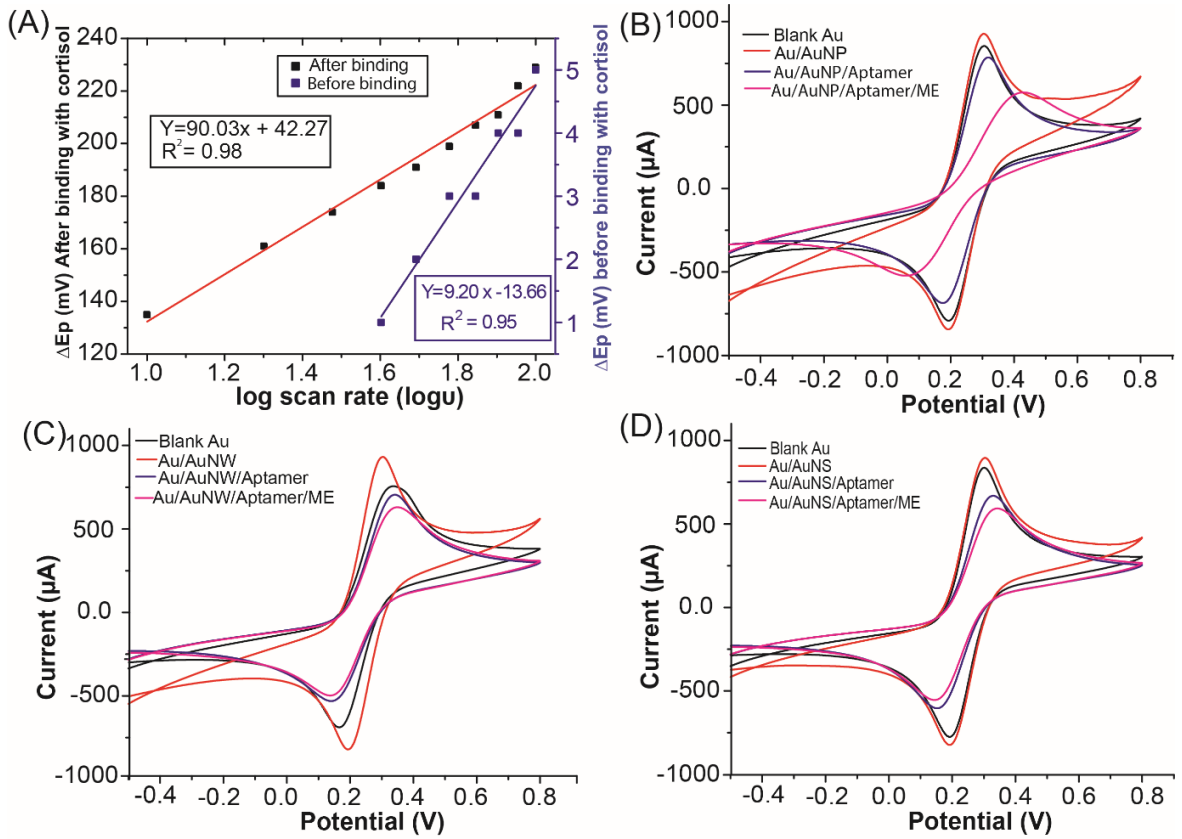


**Figure S5. Interfacial electron transfer characteristic and comparative nanomaterials sensor characterization studies. (A)** Plot of peak potential ($E_p$) vs. ln($\nu$) for redox MB before (blue) and after (black) aptamer-cortisol interaction. Characterization of various nanomaterial sensors through CV recorded by using Ag/AgCl as reference electrode and platinum wire as counter electrode and gold electrode as working with **(B)** AuNP, **(C)** AuNS, and **(D)** AuNW surface modification. Scan rate of 0.1 V/s in 10 mM potassium hexacyanoferrate solution with 0.2 M KCl prepared in PBS. The stock concentration of AuNP, AuNW and AuNS were $6.5\times10^{11}$/mL, 50 μg/mL, and $3.6\times10^{8}$/mL, respectively.

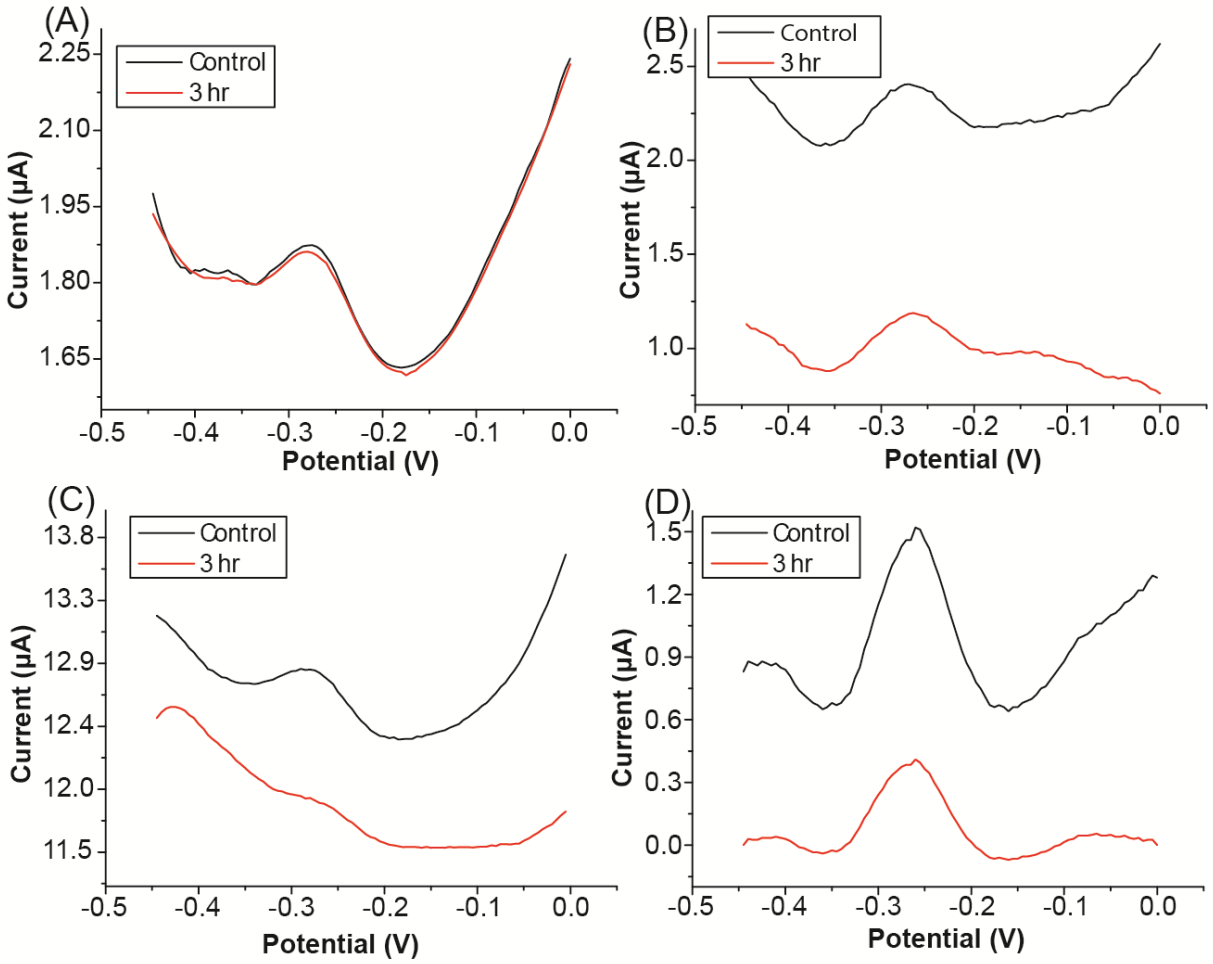


**Figure S6. Comparison of DPV voltammogram**. Measured at aptamer immobilized over **(A)** NC modified electrodes, **(B)** AuNP modified electrode, **(C)** AuNS modified electrode, and **(D)** AuNW modified electrode. Signal was recorded with aptasensor as control, and aptasensor response was recorded after incubation in serum for 3 hr at 4ºC.

**Table S1.** Composition of buffers.

| No. | Buffer name | Composition |
|---|---|---|
| 1. | Aptamer binding buffer | 50 mM Tris-HCl, 137 mM NaCl, and 5 mM $MgCl_2$, pH 7.4 |
| 2. | Measurement buffer | 1× PBS with 0.2 M KCl, pH 7.4 |

**Table S2.** Determination of sensing accuracy

| Method | Spiked concentration (µM) | Measured concentration (µM) | Signal | % Recovery* |
|---|---|---|---|---|
| **Absorbance** | 1.0 | 0.981 | 0.255 | 98.11 |
| **Fluorescence** | 1.0 | 1.021 | 36885.7 | 102 |
| **Aptasensor** | 1.0 | 0.987 | 0.652 | 98.7 |

*The % recovery value was calculated through, $C_m$-$C_0/C_a$×100, where $C_m$ is measured concentration through calibration curve, $C_0$ is sample concentration in the unspiked sample, and $C_a$ is the spiked concentration.

**References:**

(1) Tu, E.; Pearlmutter, P.; Tiangco, M.; Derose, G.; Begdache, L.; Koh, A. Comparison of Colorimetric Analyses to Determine Cortisol in Human Sweat. ACS Omega **2020**, 5 (14), 8211–8218.
(2) Sweat, M. L. Sulfuric Acid-Induced Fluorescence of Corticosteroids. Anal. Chem. **1954**, 26 (4), 773–776.