Electronic Supplementary Material (ESI) for RSC Advances.


# One-step enzyme-free dual electrochemical immunosensor for Histidine-Rich Protein 2 determination

## Supplementary Information

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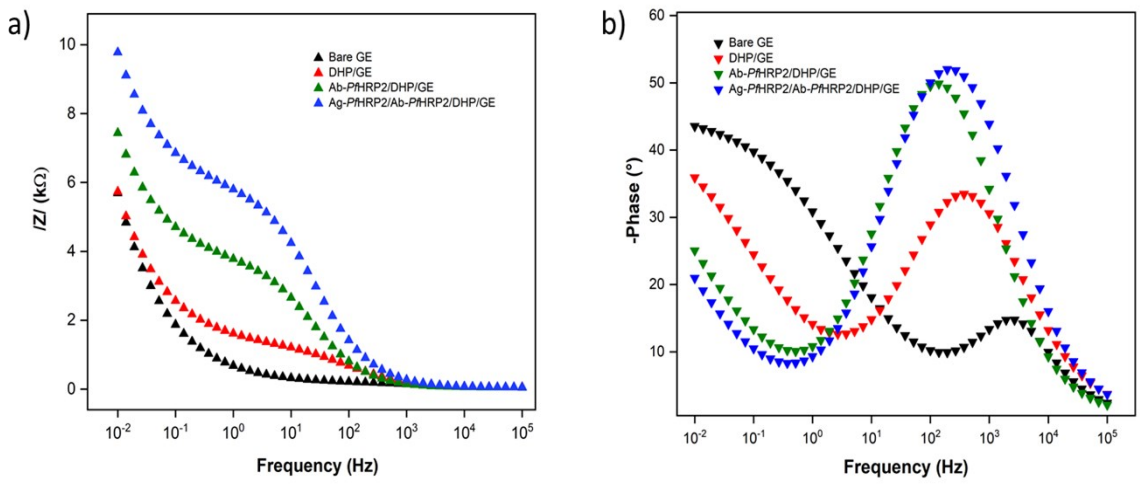


**Fig. SI 1.** Bode plot impedance a) and phase b) of the electrode surface modifications.

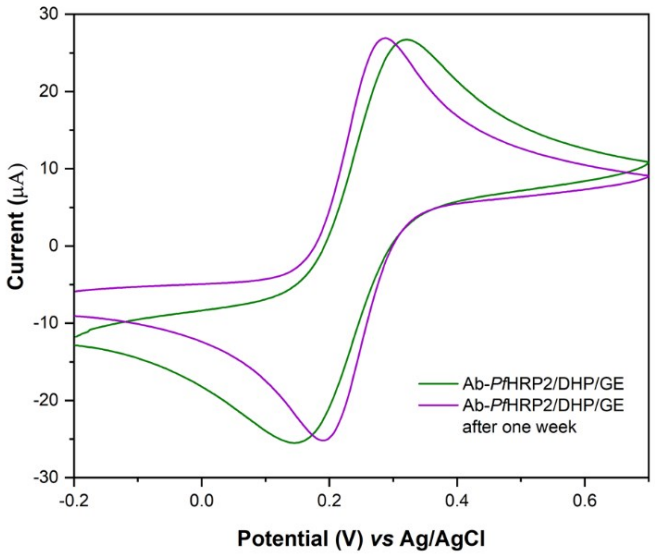


**Fig. SI 2.** Cyclic voltammograms of immunosensor before and after storage during one week at 4 °C.

## SI 3. ELISA test

ELISA 96-well microtiter plates (NUNC/Marxisorp) were sensitized for 16 hours at 4 °C in a humid chamber, with 50 µL per well of a solution containing 4 µg $mL^{-1}$ *Pf*HRP2 mice antibodies diluted in 0.05 mol $L^{-1}$ carbonate-bicarbonate buffer pH 9.6. After this period, the plates were washed with PBS containing 0.05 % Tween 20 and then blocked for 1 hour at 37 °C with PBS containing 5 % bovine serum albumin. Then, recombinant

*Pf*HRP2 protein was added in different concentrations and the resulting solutions were incubated for 1 hour at 37 °C. Afterward, the *Pf*HRP2 chicken antibodies were added at a concentration of 0.25 µg per well. These plates were incubated for 1 hour at 37 °C in a humid chamber, followed by three washes with PBS containing 0.01 % Tween 20. Next, goat anti-chicken IgG antibody conjugated with horseradish peroxidase (KPL) was added at 1/5000 dilution and incubated again for 1 hour at 37 °C in a humid chamber. After a new cycle of three washes, 50 µL of one-step reagent (SCIENCO) were added to the plate and incubated for 20 minutes at room temperature in the absence of light. The reaction was stopped with 50 µL of 1 mol $L^{-1}$ $H_2SO_4$. The generated product was quantified by reading the optical density (O.D.) at 450 nm in a spectrophotometer (FlexStation3, Molecular Devices).

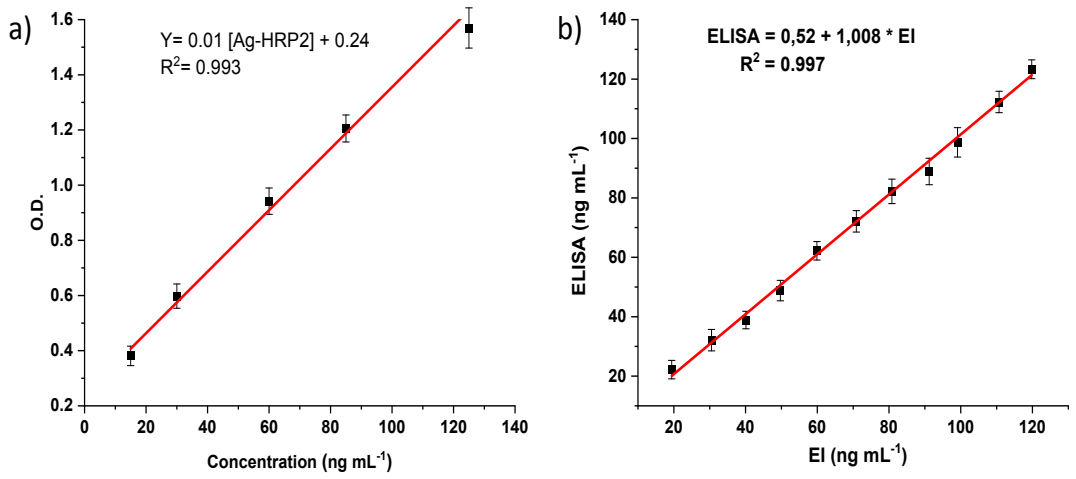


**Fig. SI 3.** a) ELISA calibration curve: Y = 0.01 [Ag-*Pf*HRP2] + 0.24, with an $R^2$ = 0.993. b) Correlation between the ELISA and the electrochemical immunosensor by EIS detection.