Supplementary Materials: Electrochemical Immunosensor for Detection of IgY in Food and Food Supplements

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1. Electrochemical Performance of the Gold NEEs

With the purpose to make the method the most reproducible possible, only NEEs that show similar CVs were used as transducers for the immunosensor preparation. To identify best working NEEs, CVs of Methylene Blue 0.1 mM in PBS 0.01 M supporting electrolyte were recorded. Figure S1 shows three CVs recorded in the same solution at different bare NEEs. The behavior of these electrodes can be consider similar in terms of current and potential both for the anodic and cathodic peaks. Data of such voltammograms are detailed in Table S1. It is worth to note that the main parameter to consider for our immunosensor in the cathodic current.

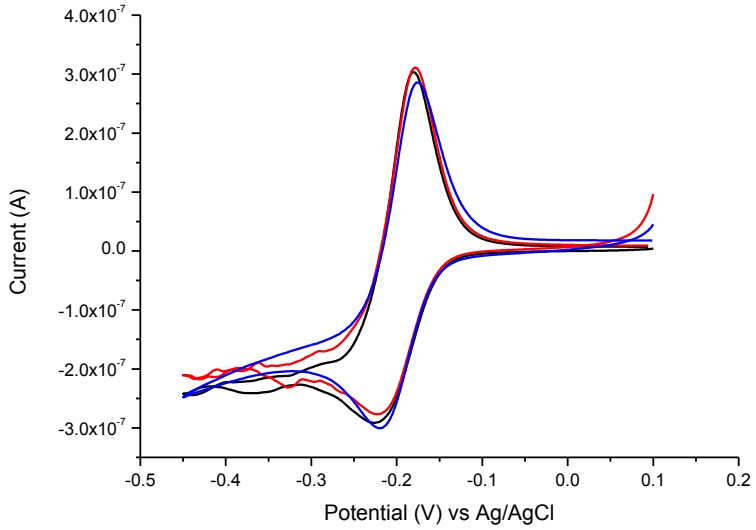

Figure S1. CVs of 0.1 mM MB in 0.01 M PBS recorded at three different bare NEEs. Scan rate: 10 mV/s.

Table S1: Comparison between peaks and currents potential of three different electrodes.

Electrode	E_c (V)	I_c (A 10^{-7})	E_a (V)	I_a (A 10^{-7})
1 (Blue CV)	−0.220	−2.823	−0.176	3.920
2 (Red CV)	−0.226	−2.685	−0.173	5.016
3 (Black CV)	−0.232	−2.875	−0.176	5.087

For more information about NEEs, see Refs. 14–16.

2. Optimization of the Concentration of the Substrate H_2O_2

The addition of 0.5 mM H_2O_2 to MB solution does not cause any change in the MB pattern recorded at a bare NEE [1]. To define the appropriate concentration of substrate, tests with increasing concentration of H_2O_2 were performed: 0.5 mM, 1.0 mM and 1.5 mM H_2O_2 where successively added to the same solution and CVs of MB in presence of the enzyme and its substrate were recorded and are shown in Figure S2.

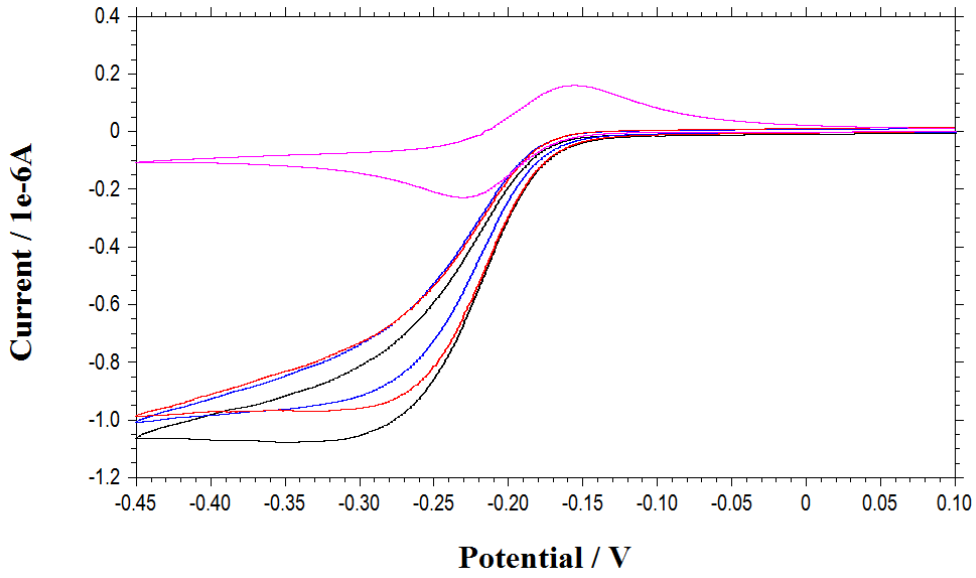

Figure S2: Cyclic voltammograms recorded at the NEE immunosensor in 0.1 mM MB, 0.01 M PBS solution solution; scan rate 10 mV/s. Black line 0.5 mM, red 1.0 mM and blue line 1.5 mM H_2O_2.

It can be observed that the lower concentration of hydrogen peroxide tested is enough to activate the electrocatalytic cycle. As shown in figure S1, after the second (red line) and third (blue line) addition of substrate, the catalytic current does not increase, in comparison to the first addition (black line), meaning that the enzyme present on the electrode surface is able to entirely react with the substrate and that god saturated. This behavior was verified for each concentration of IgY tested, from the lower to the higher, demonstrating that the concentration of redox mediator and hydrogen peroxide are optimal for our purposes.

References

1. Silvestrini, M.; Schiavuta P.; Scopece, P.; Pecchielan, G.; Moretto L.M.; Ugo P. Modification of nanoelectrode ensembles by thiols and disulfides to prevent non specific adsorption of proteins. *Electrochim Acta* **2011**, *56*, 7718–7724.