Supporting information

A unique sensing interface that allows the development of an electrochemical immunosensor for the detection of Tumor Necrosis Factor α in whole blood

Cheng Jiang, Muhammad Tanzirul Alam, Saimon Moraes Silva, Safura Taufik, Sanjun Fan, J. Justin Gooding*

School of Chemistry, Australian Centre for NanoMedicine and ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, The University of New South Wales, Sydney, NSW 2052, Australia

Corresponding Author

J. Justin Gooding: Justin.Gooding@unsw.edu.au

Tel: +61-2 9385 5384.

Electrochemical Response to Hydrogen Peroxide at the Immunosensor. The electrocatalytical reactivity of the final immunosensor towards H_2O_2 was investigated by cyclic voltammetry. Figure. S-1(a) depicts the current response in the absence and presence of H_2O_2. A pair of redox peaks were observed in the blank substrate solution, which contributes to the redox reaction of ferrocene. It was observed an increase of both the oxidation current and reduction current upon the addition of H_2O_2, which is in accordance with literature report of Fc mediated HRP catalytic reaction toward H_2O_2.[1-3] This result implies that an enzyme-dependent catalytic current response of H_2O_2 which originates from the HRP reaction and the soluble redox mediator of ferrocenemethanol could effectively shuttle electrons from the base electrode.

This typical enzyme-dependent catalytic process (shown in Scheme. 1) can be expressed as follows:

H_2O_2 + HRP (red) $\longrightarrow$ H_2O+ HRP (ox)

HRP (ox) +Fc $\longrightarrow$ HRP (red) + Fc^+

$Fc^+ + e^- \longrightarrow$ Fc

First, H_2O_2 in the solution is reduced by the immobilized HRP. Then the reduced HRP is regenerated with the aid of the mediator (Fc), while Fc itself is oxidized in the enzymatic reaction. Finally, the oxidized Fc is electrochemically reduced on the electrode, leading to an increase of the reduction current. Since concentration of TNF-α influences the immobilized amount of HRP, which is further related to reductive current produced, thus a methodology depends on the relationship between concentration of TNF-α and reductive current signal can be developed to monitor TNF-α.

Influence of H_2O_2 on the response of immunosensor is of great importance, the amperometric response of the mixed layers based immunosensor for H_2O_2 reduction was thus examined at an applied potential of 0.05 V upon successive addition of H_2O_2 in a gentle stirring PBS containing 0.1 mM ferrocenemethanol. As illustrated in Figure. S-1(b) the immunosensor displays increasing amperometric responses to H_2O_2

with the linear ranges from 0.01 mM to 1.07 mM and the maximal cathodic peak current change can be obtained at ~2.0 mM H_2O_2 and then it started to reach a plateau. In order to avoid the irreversible transition of the HRP to its higher oxidized and inactive form at higher H_2O_2 concentration,[4] 2.0 mM of H_2O_2 was chosen for following detection.

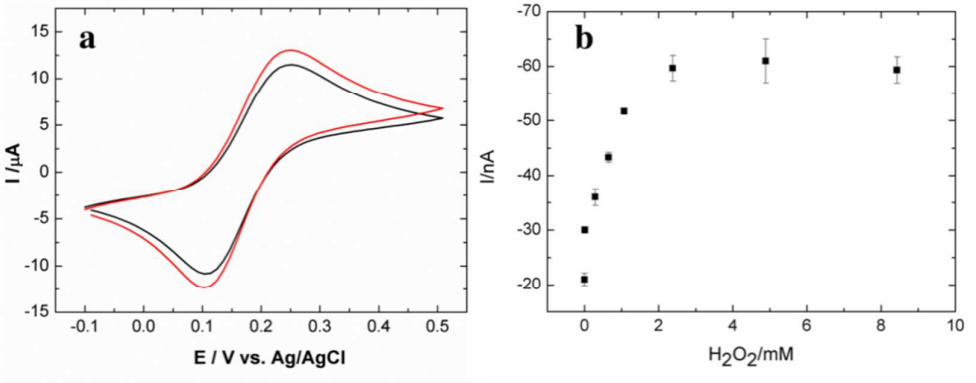

Figure S-1. (a) Cyclic voltammograms of the final immunosensor at 0.1 mM Fc, pH 7.4 PBS in the absence (black line) and presence of 50 μM H_2O_2 (red line) with a scan rate of 20 mV/s. (b) Dependence of the chronoamperometric reduction current on the concentration of H_2O_2 for the PPC-PBA mixed layers-based immunosensor in in pH 7.4 PBS containing 0.1 mM Fc at an applied potential of 0.05 V *vs.* Ag/AgCl. Error bars represent the standard deviation, n=3.

References

1. Camussi, G.; Albano, E.; Tetta, C.; Bussolino, F. The molecular action of tumor necrosis factor - α . *Eur. J. Biochem.* **1991,** *202* (1), 3-14.
2. Chen, X.; Chang, J.; Deng, Q.; Xu, J.; Nguyen, T. A.; Martens, L. H.; Cenik, B.; Taylor, G.; Hudson, K. F.; Chung, J. Progranulin does not bind tumor necrosis factor (TNF) receptors and is not a direct regulator of TNF-dependent signaling or bioactivity in immune or neuronal cells. *J. Neurosci.* **2013,** *33* (21), 9202-9213.
3. Sun, Z.; Deng, L.; Gan, H.; Shen, R.; Yang, M.; Zhang, Y. Sensitive immunosensor for tumor necrosis factor α based on dual signal amplification of ferrocene modified

self-assembled peptide nanowire and glucose oxidase functionalized gold nanorod. *Biosens. Bioelectron* **2013,** *39* (1), 215-219.
4. Mainz, E. R.; Serafin, D. S.; Nguyen, T. T.; Tarrant, T. K.; Sims, C. E.; Allbritton, N. L. Single Cell Chemical Cytometry of Akt Activity in Rheumatoid Arthritis and Normal Fibroblast-like Synoviocytes in Response to Tumor Necrosis Factor α. Anal. Chem. 2016. 88 (15), 7786–7792.