Supporting Information

Enzyme-free electrochemical immunosensor based on methylene blue and the electro-oxidation of hydrazine on Pt nanoparticles

Gorachand Dutta[a], Sureshbabu Nagarajan[b, c], Lisa J. Lapidus[b], Peter B. Lillehoj[a, *]

[a]Department of Mechanical Engineering, Michigan State University, East Lansing, MI 48824, USA

[b]Department of Physics and Astronomy, Michigan State University, East Lansing, MI 48824, USA

[c]Centre for Drug Discovery and Development, Sathyabama University, Chennai, Tamil Nadu 600119, India

[*]Corresponding author: E-mail: lillehoj@egr.msu.edu

Preparation of MB-conjugated anti-*Pf*HRP2 IgG

A solution of MB succinimidyl ester was prepared by dissolving 1 mg/mL in DMSO. The covalent conjugation of MB to the lysine side chains of the IgG antibody was initiated by adding 300 µL of the MB succinimidyl ester solution to 3 mL of IgG solution (~330 µg/mL) in 20 mM HEPES, pH 8.0. The molar ratio of MB to IgG antibody was ~30:1. The reaction was carried out overnight at 4°C with gentle agitation. Excess (unbound) MB in the solution was removed by dialysis for 24 hours at 4°C through a 3 kDa membrane. MB-conjugated IgG was concentrated to 1 mL by passage through a 3 kDa cut-off membrane. The antibody complex was characterized by SDS-PAGE. Solutions of IgG and MB-conjugated IgG were loaded onto the SDS gel. The appearance of blue bands for the MB-conjugated IgG complex in the absence of Coomassie Blue staining confirmed MB binding to IgG (Fig. S1a). The bands at 24 and 50 kDa correspond to the light and heavy chains, respectively, and bands at 72, 96 and 102 kDa correspond to the combinations of light and heavy chains.

Comparison between the IgG and MB-conjugated IgG complex clearly shows the differences in the pattern of the bands. For IgG, the bands at lower molecular weights (24 and 50 kDa) are more intense while the bands at higher molecular weights are less intense when compared to the MB-conjugated IgG complex (Fig. S1b). Further, these results show differences in the molecular weight of all the bands due to MB conjugation with IgG.

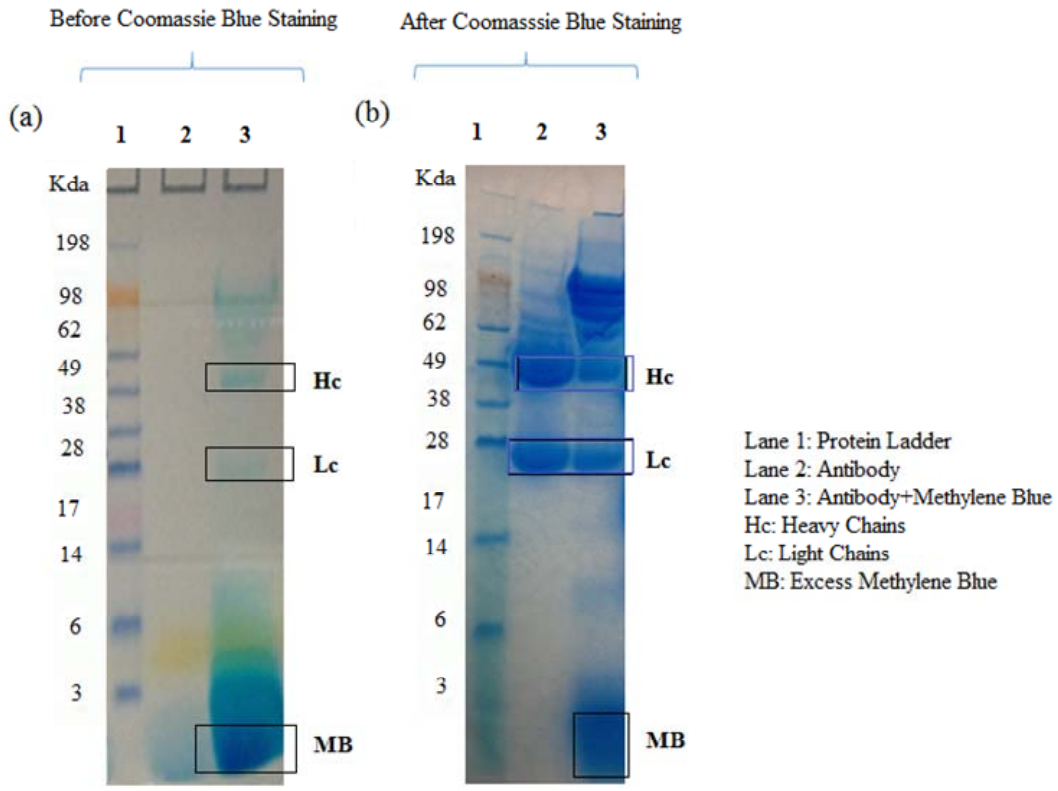

Fig. S1. SDS-PAGE analysis of IgG and MB-conjugated IgG complex before (a) and after (b) Coomassie Blue staining. IgG consists predominantly of two bands corresponding to light (MW~24 kDa) and heavy chains (MW~50 kDa), whereas the higher molecular weight bands (72-102 kDa) correspond to the MB-conjugated IgG complex.

Electrode surface characterization after protein immobilization

Atomic force microscopy (AFM) scans were performed on APTES-glutaraldehyde modified ITO electrodes after subsequent immobilization of anti-*Pf*HRP2 IgM, *Pf*HRP2 antigen, and MB-conjugated anti-*Pf*HRP IgG. These scans show a distinct change in surface morphology after each immobilization step. Specifically, the adsorption of the IgM antibody on the electrode surface is represented by ellipsoid features 4-6 nm in height (Fig S2a). There are also a few larger particles which is likely due to the precipitation of IgM. The binding of *Pf*HRP2 antigen to the IgM-coated surface results in an increase in height of the ellipsoid features to 10-12 nm (Fig. S2b). The third immobilization step results in binding of MB-conjugated IgG to the protein complex which further increases the height of the features to 18-22 nm (Fig. S2c). These results are consistent with previous reports on antibody-antigen surface immobilization (Ouerghi et al., 2002).

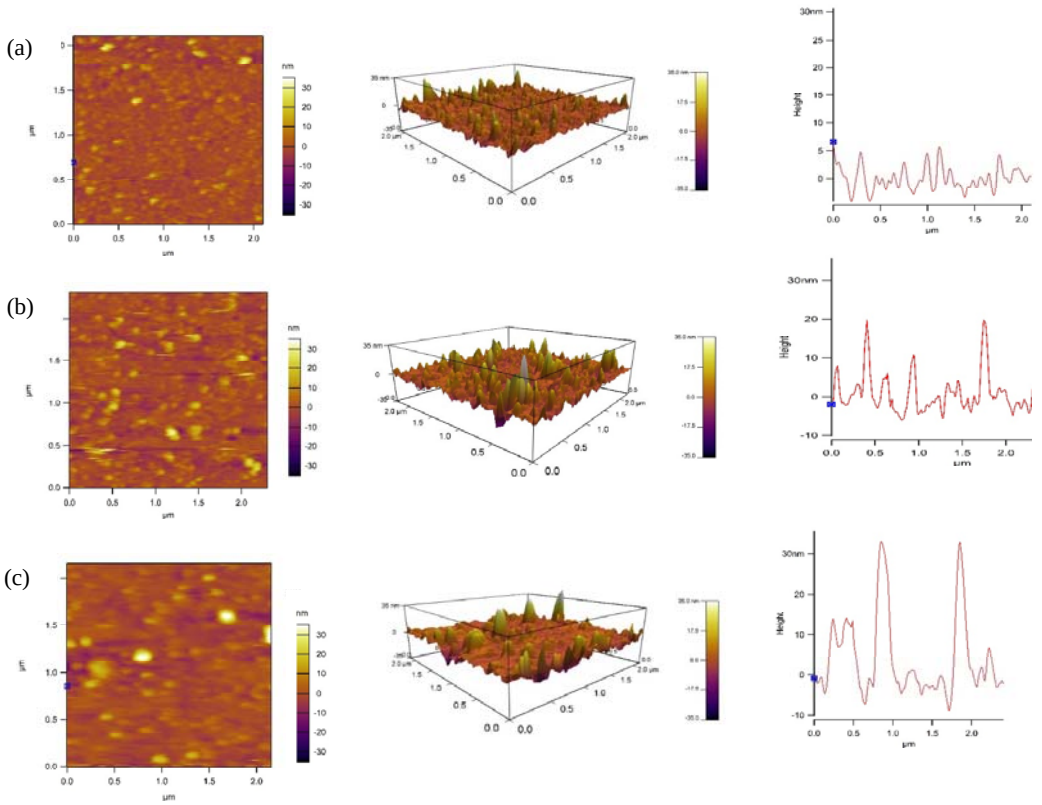

Fig. S2. AFM tapping mode images of APTES-glutaraldehyde modified ITO electrodes after sequential immobilization of (a) anti-*Pf*HRP2 IgM (100 µg/mL), (b) *Pf*HRP2 antigen (10 ng/mL in PBS), and (c) MB-conjugated anti-*Pf*HRP2 IgG (10 µg/mL). The scan size is 2 µm × 2 µm and the z-scales for the 2D and 3D scans are 60 nm and 70 nm, respectively.

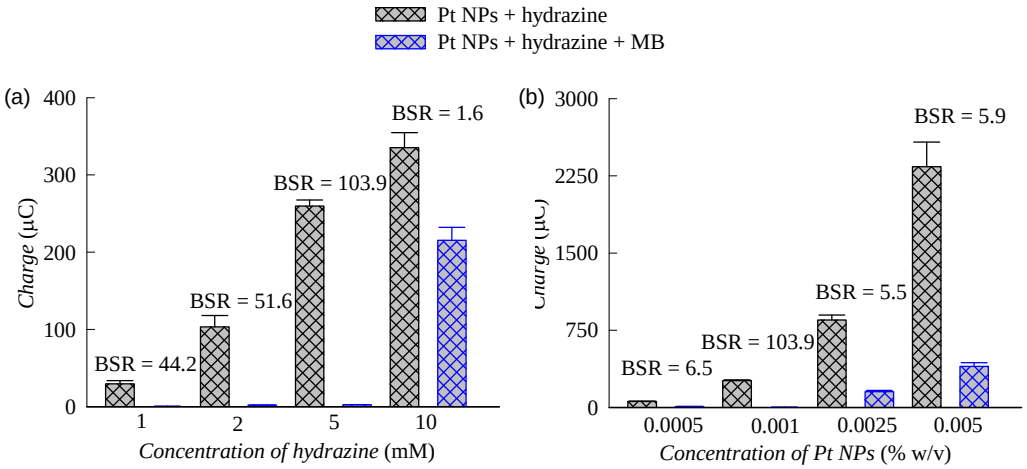

Fig. S3. (a) Chronocoulometric charges of hydrazine solutions containing varying concentrations of hydrazine and 0.001% (w/v) Pt NPs with and without 10 μM MB. (b) Chronocoulometric signals of hydrazine solutions containing 5 mM hydrazine and varying concentration of Pt NPs with and without 10 μM MB. Signals are taken at 50 s from chronocoulograms obtained at 0.05 V (*vs* Ag/AgCl). Each bar represents the mean ± SD of three separate measurements using new electrodes.

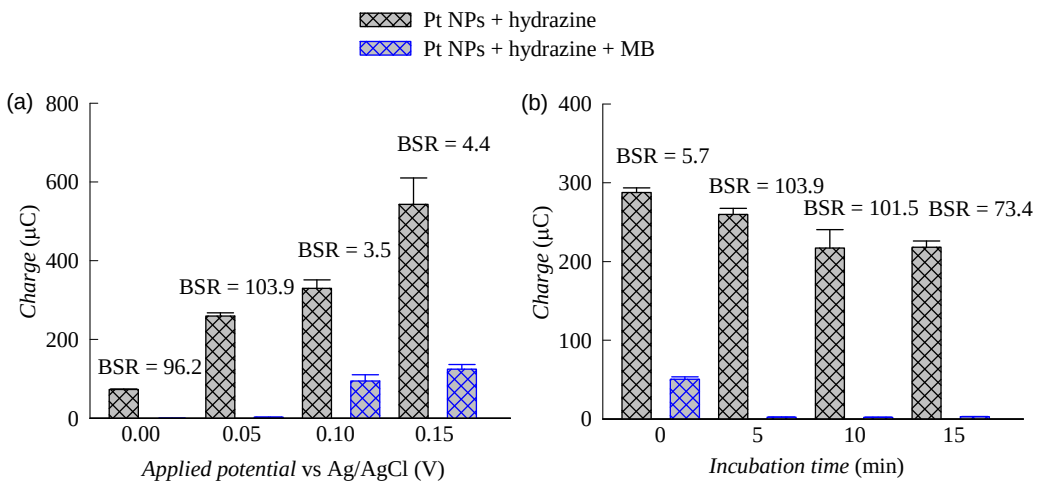

Fig. S4. (a) Chronocoulometric signals of hydrazine solutions containing 5 mM hydrazine and 0.001% (w/v) Pt NPs with and without 10 μM MB at varying bias potentials. (b) Chronocoulometric signals of hydrazine solutions containing 5 mM hydrazine and 0.001% (w/v) Pt NPs with and without 10 μM MB at 0.05 V (*vs* Ag/AgCl) at varying incubation time. Charges are taken at 50 s from chronocoulograms. Each bar represents the mean ± SD of three separate measurements using new electrodes.

Determination of the lower limit of detection

The lower limit of detection was calculated based on the method described in *Quantitative Chemical Analysis* (Harris 2007). Briefly, we first determined 3× the standard deviation (SD) of the signal at zero concentration which is represented by the dashed line in Figs. 4b and 5c. The limit of detection was taken as the lowest detectable concentration below this +3SD line. If the concentration profile included data points above the +3SD line, the limit of detection was calculated as the intersection between the +3SD and linear correlation line.

References

Harris D.C., 2007. *Quantitative Chemical Analysis*, seventh ed. Quality Assurance and Calibration Methods. Craig Bleyer, New York, pp. 84–87.

Ouerghi O., Touhami A., Othmane A., Ouada H. B., Martelet C., Fretigny C., Jaffrezic-Renault N., *Sens. Actuators B* **84**, 2002, 167–175.