Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Ultrasensitive enzyme-free electrochemical immunosensor based on redox cycling amplification using methylene blue

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Preparation of MB-labeled secondary antibody

MB was conjugated with anti-*Pf*HRP2 IgG by coupling the amine groups of IgG and active ester group of MB as previously reported[1] with minor modifications. 1 mg/mL of MB succinimidyl ester solution was prepared in DMSO. The covalent conjugation of MB to the lysine side chains of IgG 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 MB to IgG molar ratio was ~30:1. The resulting solution was incubated overnight at 4°C with gentle agitation/rotation. Excess (unconjugated) MB was removed by centrifugation for 30 min at 10,000 rpm. MB-labeled antibody was diluted in 1 mL of PBS and stored at 4°C prior to use.

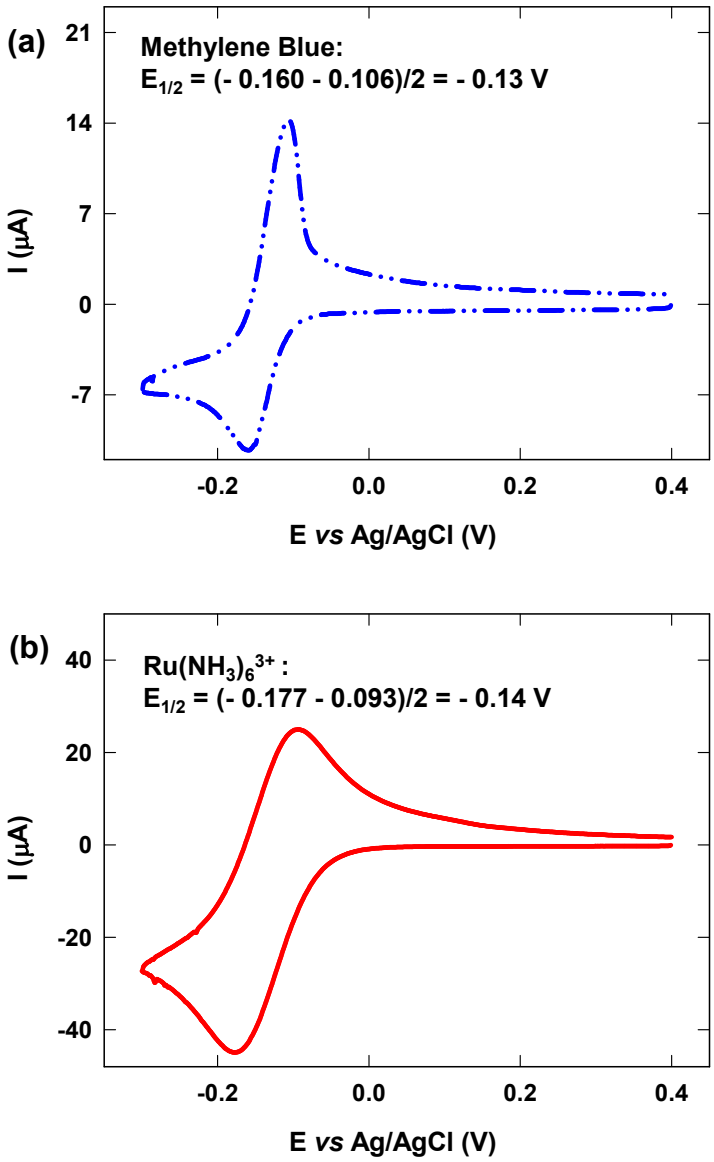

Fig. S1 Estimated formal potentials (*vs* Ag/AgCl) of the redox couples MB_{ox}/MB_{red} (a), and $Ru(NH_3)_6^{3+}/Ru(NH_3)_6^{2+}$ (b) calculated from voltammograms.

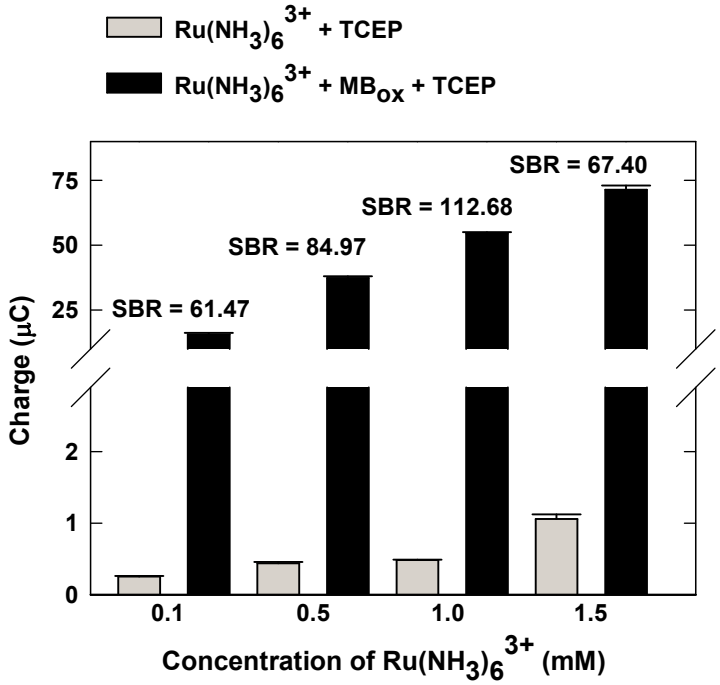

Fig. S2 Chronocoulometric signals obtained from solutions containing varying concentrations of $Ru(NH_3)_6^{3+}$ and 2 mM of TCEP in PBS with and without 10 μM MB using ITO electrodes. Charges are taken at 100 sec from chronocoulograms. Each bar represents the mean ± SD of three separate measurements using new sensors.

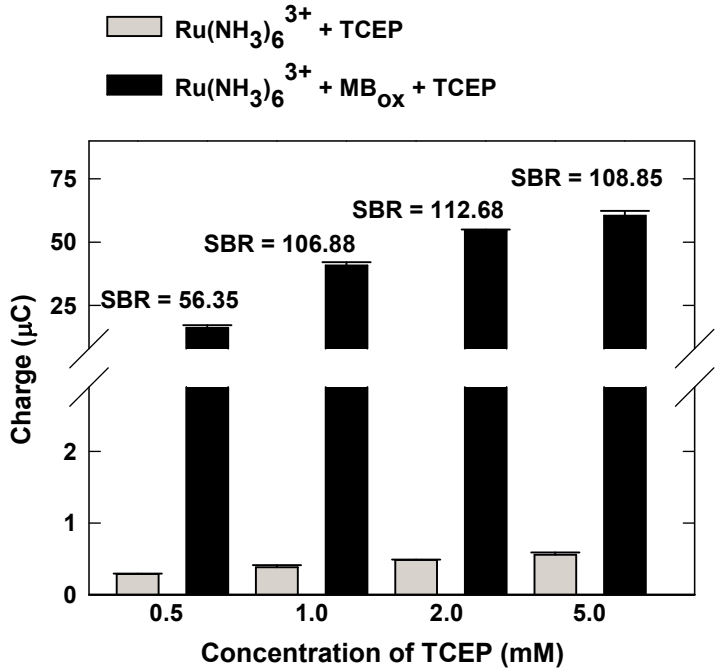

Fig. S3 Chronocoulometric signals obtained from solutions containing varying concentrations of TCEP and 1 mM of $Ru(NH_3)_6^{3+}$ in PBS with and without 10 μM of MB using ITO electrodes. Charges are taken at 100 sec from chronocoulograms. Each bar represents the mean ± SD of three separate measurements using new sensors.

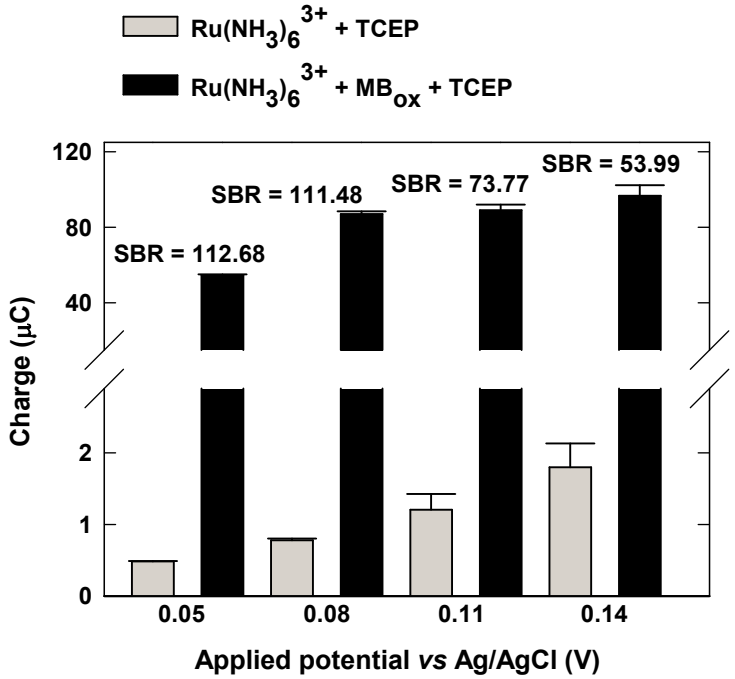

Fig. S4 Chronocoulometric signals obtained from solutions containing 1 mM of $Ru(NH_3)_6^{3+}$ and 2 mM of TCEP in PBS with and without 10 μM of MB at varying bias potentials using ITO electrodes. Charges are taken at 100 sec from chronocoulograms. Each bar represents the mean ± SD of three separate measurements using new sensors.

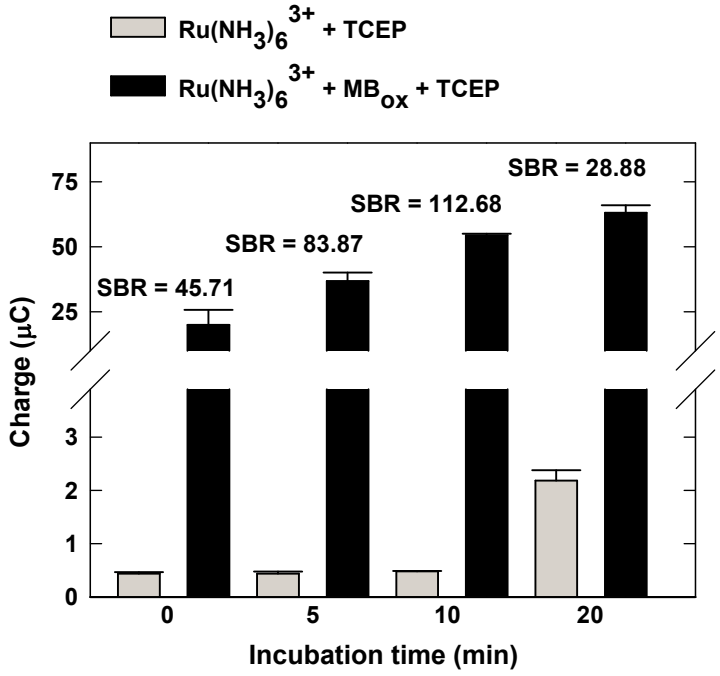

Fig. S5 Chronocoulometric signals obtained from solutions containing 1 mM of $Ru(NH_3)_6^{3+}$ and 2 mM of TCEP in PBS with and without 10 μM of MB at varying incubation time using ITO electrodes. Charges are taken at 100 sec from chronocoulograms. Each bar represents the mean ± SD of three separate measurements using new sensors.

References

1. G. Dutta, S. Nagarajan, L. J. Lapidus and P. B. Lillehoj, *Biosens. Bioelectron.*, 2017, **92**, 372.