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Supporting Information

Hemoglobin on Phosphonic Acid Terminated Self-assembled Monolayers Modified Planar Gold Electrode: Immobilization, Direct Electrochemistry and Electrocatalysis

Yu Chen,[a] Bo Jin,[b] Li-Rong Guo,[a] Xiao-Jing Yang,[a] Wei Chen,[b] Gang Gu,[b] Li-Min Zheng,*[a] and Xing-Hua Xia*[b]

[a]*State Key Laboratory of Coordination Chemistry, Coordination Chemistry Institute, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, P. R. China*

[b]*Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, P. R. China*

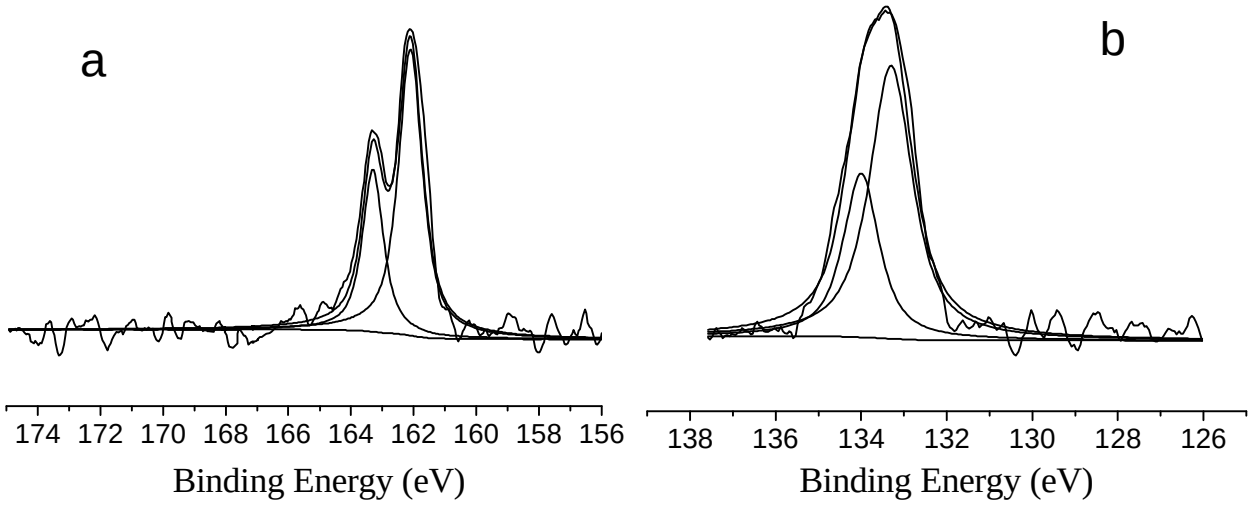

Figure S1. XPS spectra of MPPA/Au in the a) S(2p) and b) P(2p) region.

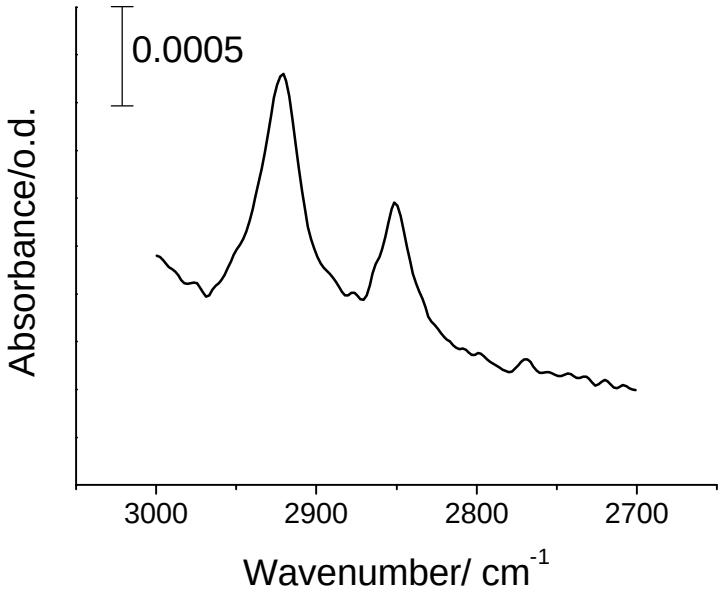

Figure S2. Fourier-transform infrared external reflection spectrum of MPPA/Au electrode was collected on a Bruker Tensor 27 (Bruker, Germany) equipped with a liquid nitrogen cooled mercury-cadmium-telluride (MCT) detector. P-polarized light beam was used. The spectrum of a clean gold surface was used as the reference over 128 scans and then the sample spectrum of the MPPA/Au surface was calculated.

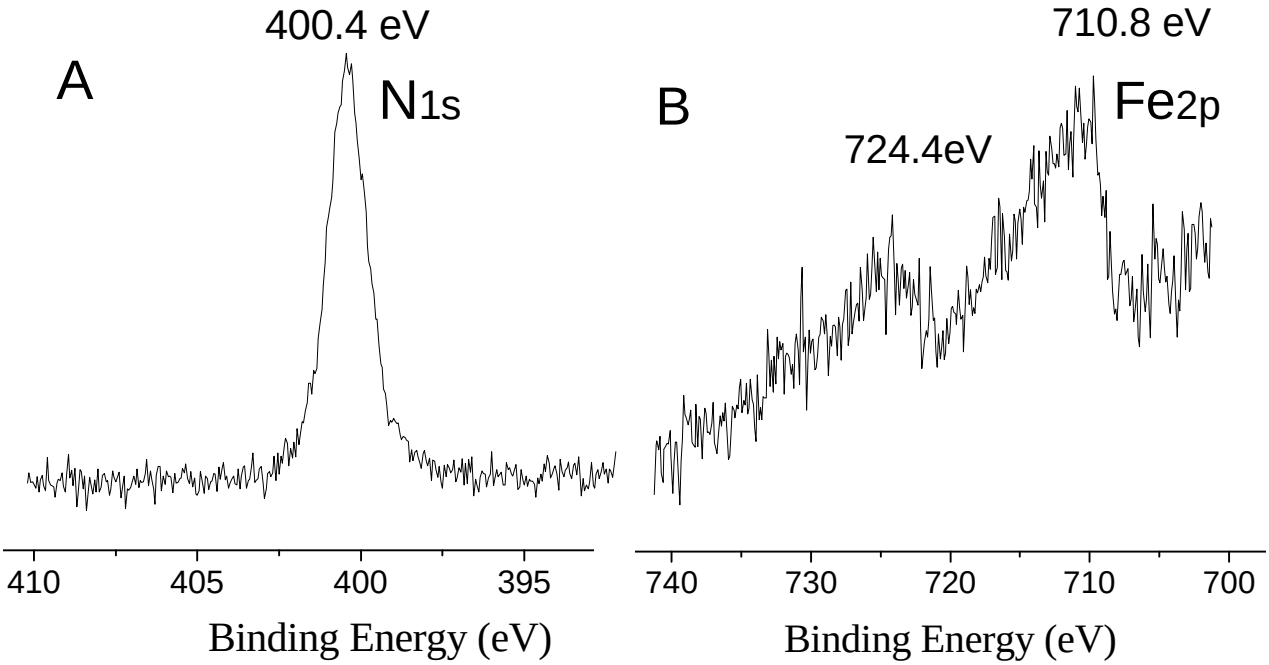

Figure S3. XPS spectra of Hb/MPPA/Au in the A) N(1s) and B) Fe(2p) region.

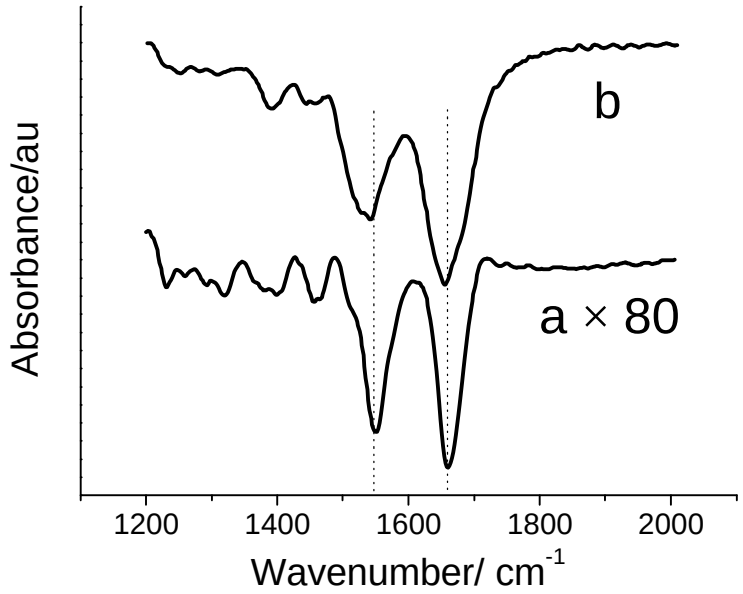

Figure S4. a) Surface-enhanced infrared absorption spectrum of Hb immobilized on MPPA/Au electrode and b) absorption infrared (IR) spectrum of native Hb.

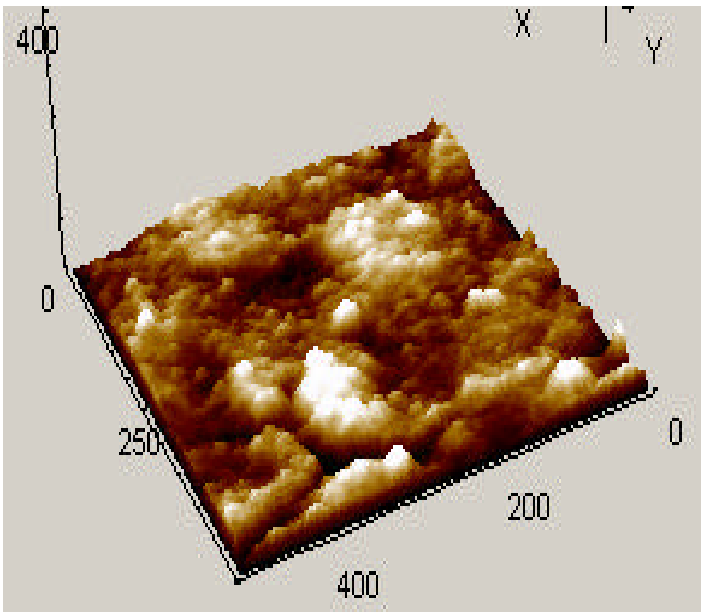

Figure S5. AFM images of Hb/MPPA/Au obtained after MPPA/Au electrode is immersed in Hb solution for 30 s.

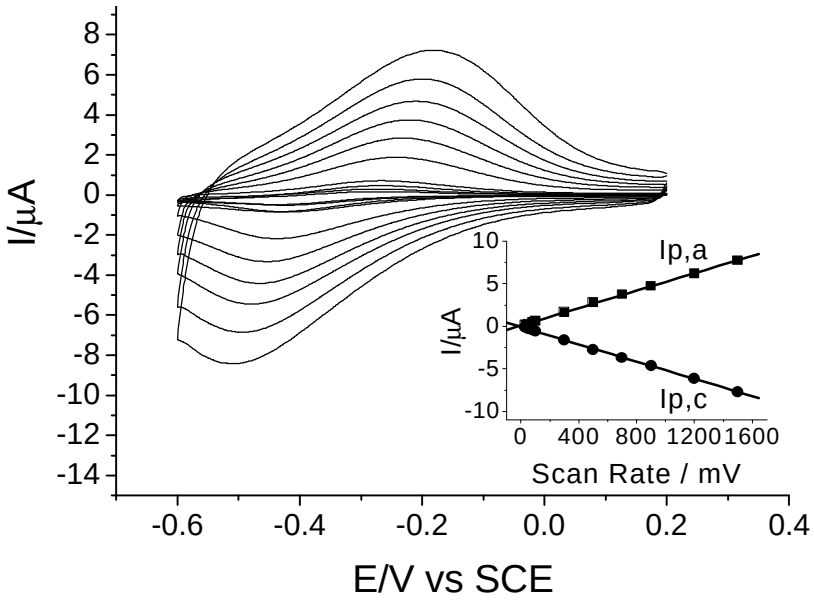

Figure S6. Cyclic voltammograms of Hb/MPPA/Au electrode in a 4.4 mM PBS (pH 7.0) at different scan rate of 30, 50, 80, 100, 300, 500, 700, 900, 1000, 1200, 1500 mVs^{-1} from inner to outer, respectively. Insert: plots of the corresponding cathodic and anodic peak currents vs. scan rate.

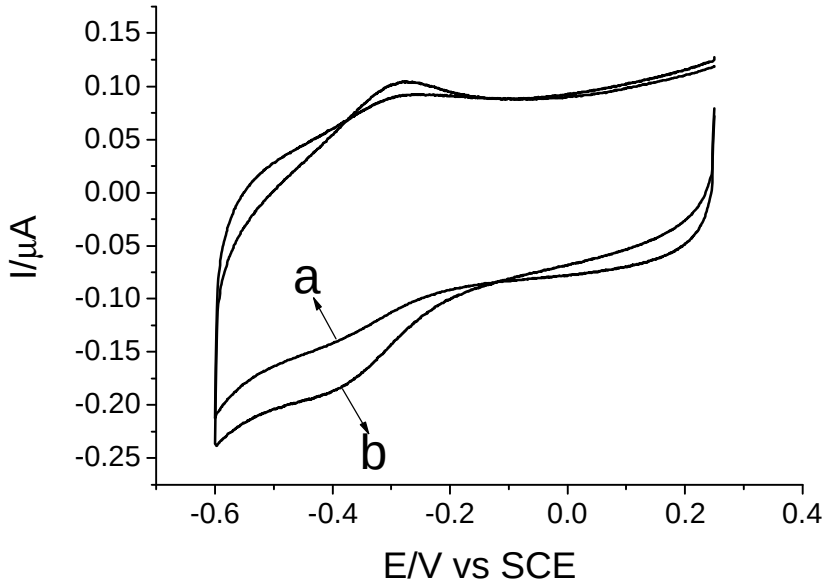

Figure S7. Cyclic voltammograms of a) MPPA/Au and b) hemin/MPPA/Au electrode in 4.4 mM PBS (pH 7.0) at a scan rate of 100 mVs^{-1} After MPPA/Au electrode was immersed 10 mM NaAc-HAc buffer (pH 5.4) containing saturated hemin solution in the dark for 20 hours at 4 °C, the hemin/MPPA/Au electrode was obtained.

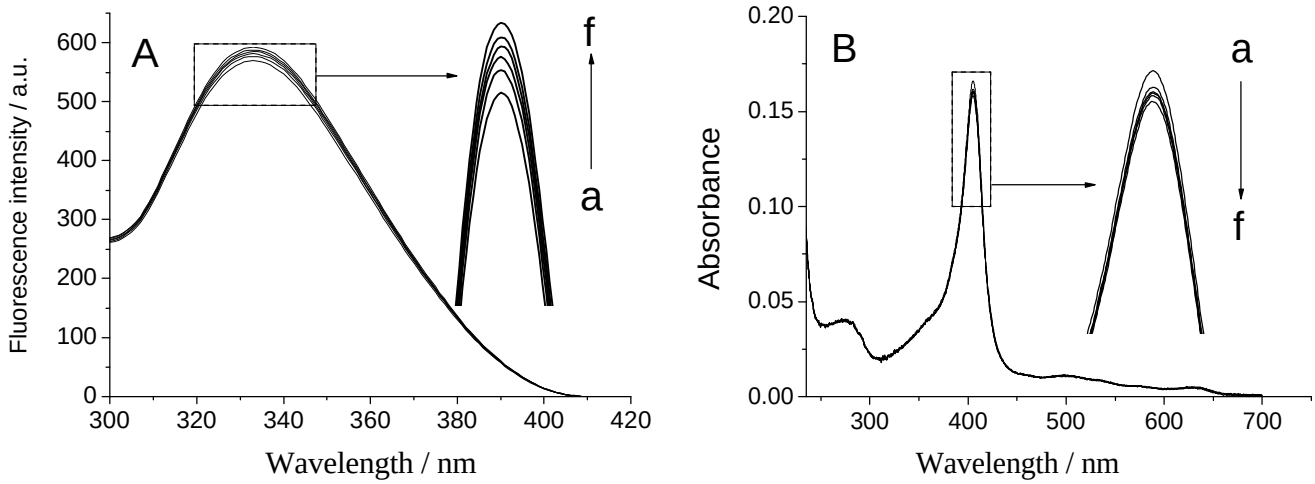

Figure S8. A) Fluorescence spectrum of 1.33 μM Hb in a) 0.01 M NaAc/HAc buffer (pH 5.4) system, as a function of H_2O_3P-$(CH_2)_4$-PO_3H_2 concentration in the range of 1.33μM ~50.5 μM: b) 1.33 μM, c) 3.99 μM, d) 10.64 μM, e) 23.9 μM and f) 50.5 μM. B) UV-vis absorption spectra of 0.266 μM Hb in a) 0.01 M NaAc/HAc buffer (pH 5.4) system, as a function of H_2O_3P-$(CH_2)_4$-PO_3H_2 concentration in the range of 1.33μM ~ 85.3μM: b) 1.33 μM, c) 2.66 μM, d) 5.32 μM, e) 18.2 μM and f) 85.3 μM.

As the data shown, the tryptophan residues fluorescence intensity of Hb increase slightly with the increasing concentration of H_2O_3P-$(CH_2)_4$-PO_3H_2, which indicates that H_2O_3P-$(CH_2)_4$-PO_3H_2 can bind to Hb protein through -PO_3H_2 groups. On the other hand, after H_2O_3P-$(CH_2)_4$-PO_3H_2 of 340 times higher concentration than that of Hb were added to the Hb-NaAc/HAc solution, the UV-vis absorption spectrum of Hb do not show any significant spectral change except a very slight decrease in intensity of the soret band is observed. Although the number of binding sites are not clear, this result of UV-vis indicates that all four heme groups bound to hemoglobin are not directly attacked and degraded by -PO_3H_2 groups of H_2O_3P-$(CH_2)_4$-PO_3H_2. According to results of fluorescence and UV-vis absorption spectrum measurement, the possibility of release of iron ion was ruled out in the presence of -PO_3H_2 groups.

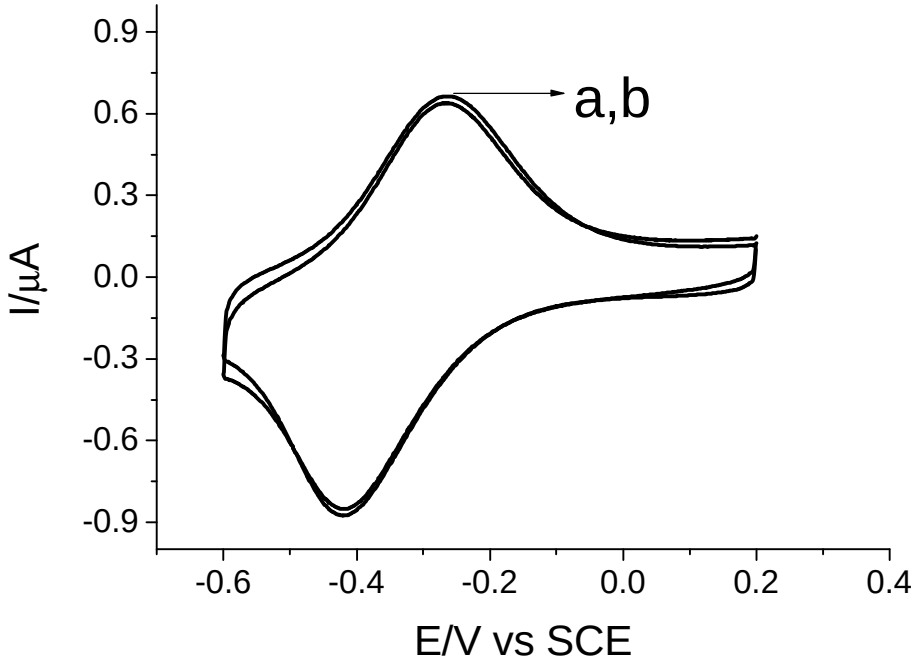

Figure S9. Cyclic voltammograms of Hb/MPPA/Au electrode a) without treatment and b) after treatment with 1M KCl solution solution for 15 h in 4.4 mM PBS (pH 7.0) at a scan rate of 100 mVs^{-1}.

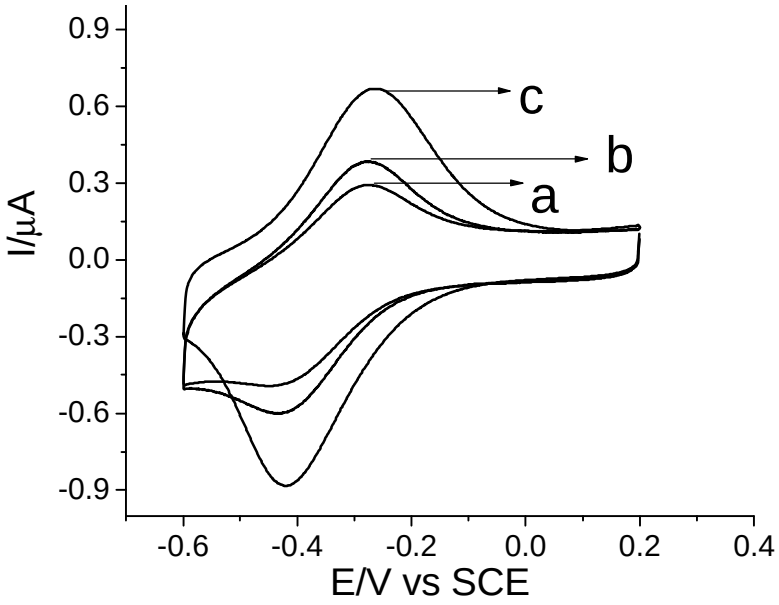

Figure S10. Cyclic voltammograms of Hb/MPPA/Au electrode obtained form 3$mgmL^{-1}$ Hb solution containing a) 4.4 mM PBS (pH 5.4), b) 0.1 M NaAc (pH 7.2) and c) 10 mM NaAc-HAc buffer (pH 5.4) in

a 4.4 mM PBS (pH 7.0) at a scan rate of 100 mVs^{-1}.

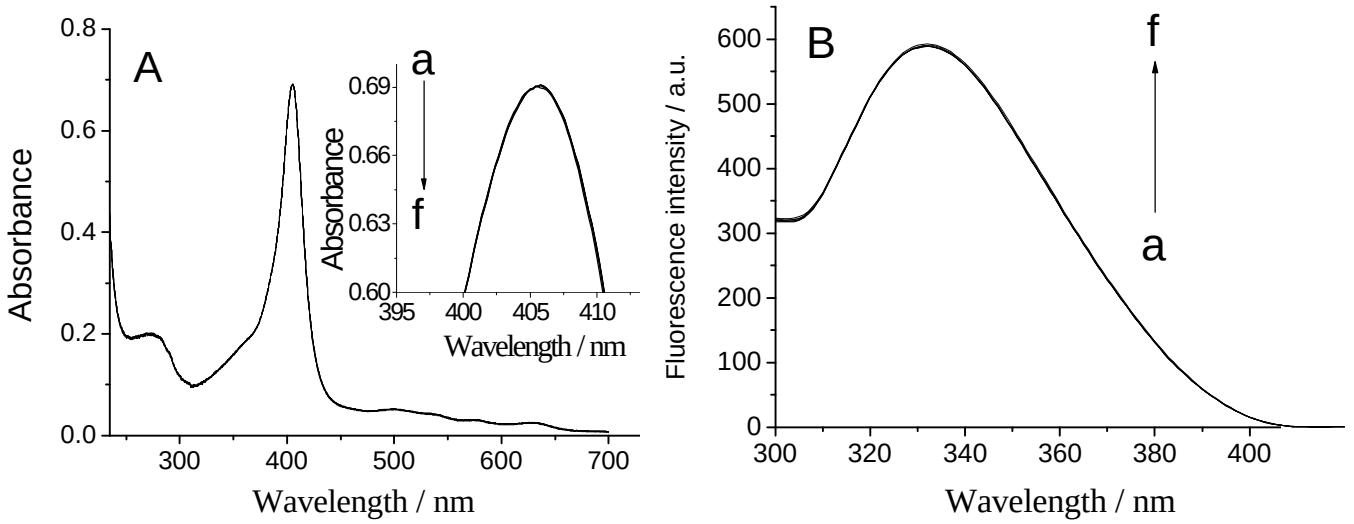

Figure S11. A) UV-vis absorption spectra of 1.33 μM Hb in Hb-NaAc-HAc buffer (5.4) system. NaAc-HAc concentrations in NaAc-HAc buffer (5.4) system: a) 0, b) 0.133, c) 0.665, d) 5.85, e) 13.3 and f) 133.3 μM. B) Fluorescence spectrum of 1.33μM Hb in Hb-NaAc-HAc buffer (5.4) system. NaAc-HAc concentrations in NaAc-HAc buffer (5.4) system: a) 0, b) 0.133, c) 0.665, d) 5.85, e) 13.3 and f) 133.3μM.

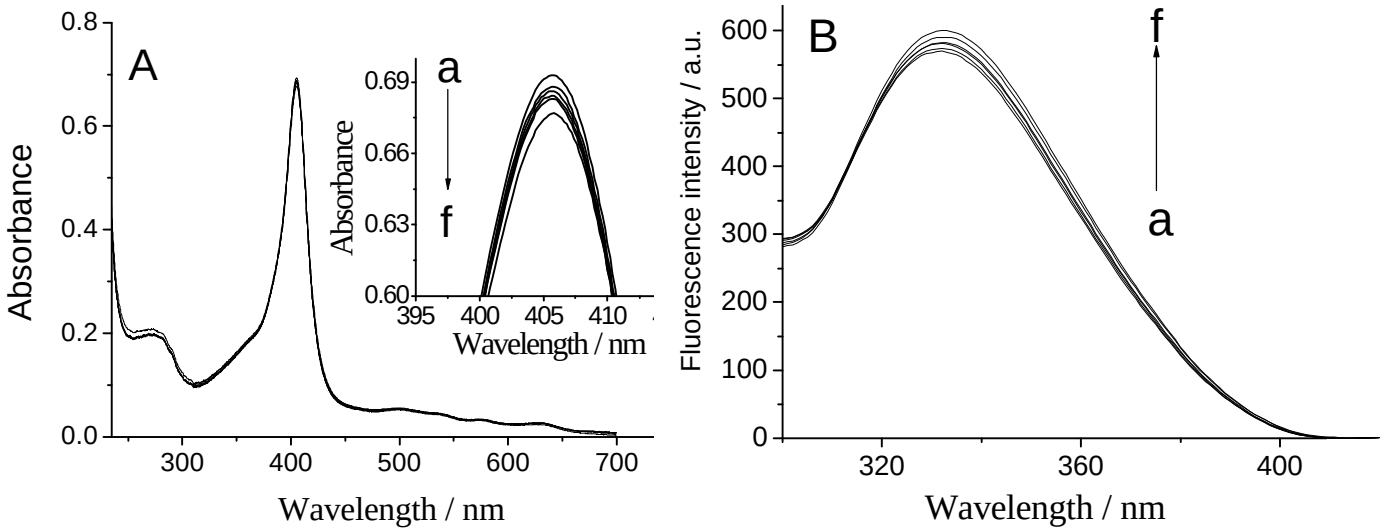

Figure S12. A) UV-vis absorption spectra of 1.33 μM Hb in Hb-PBS (5.4) system. PBS concentrations in PBS buffer (5.4) system: a) 0, b) 0.133, c) 0.665, d) 5.85, e) 13.3 and f) 133.3 μM. B) Fluorescence spectrum of 1.33 μM Hb in Hb-PBS (5.4) system. PBS concentrations in PBS buffer (5.4) system: a) 0, b) 0.133, c) 0.665, d) 5.85, e) 13.3 and f) 133.3μM.

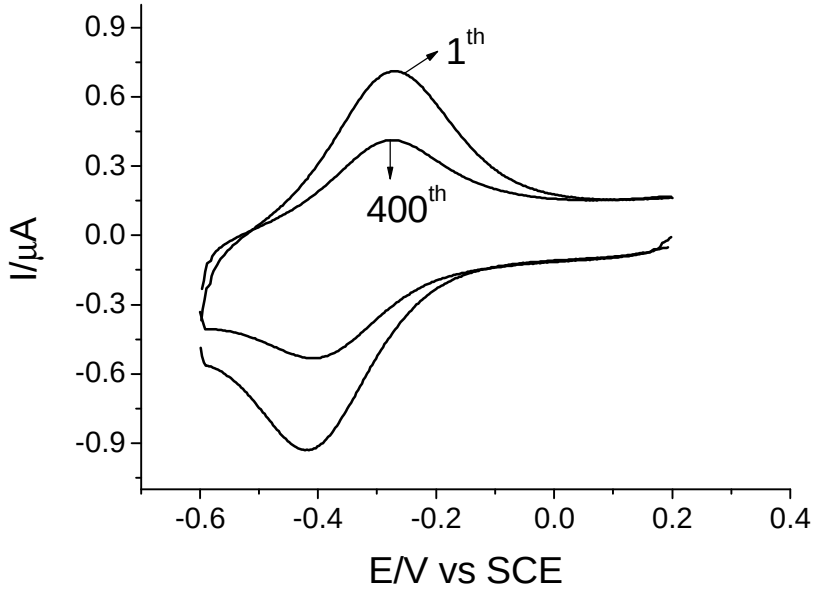

Figure S13. First and 400th recorded cyclic voltammograms of Hb/MPPA/Au in 4.4 mM PBS (pH 7.0) at a scan rate of 100 mVs^{-1}.

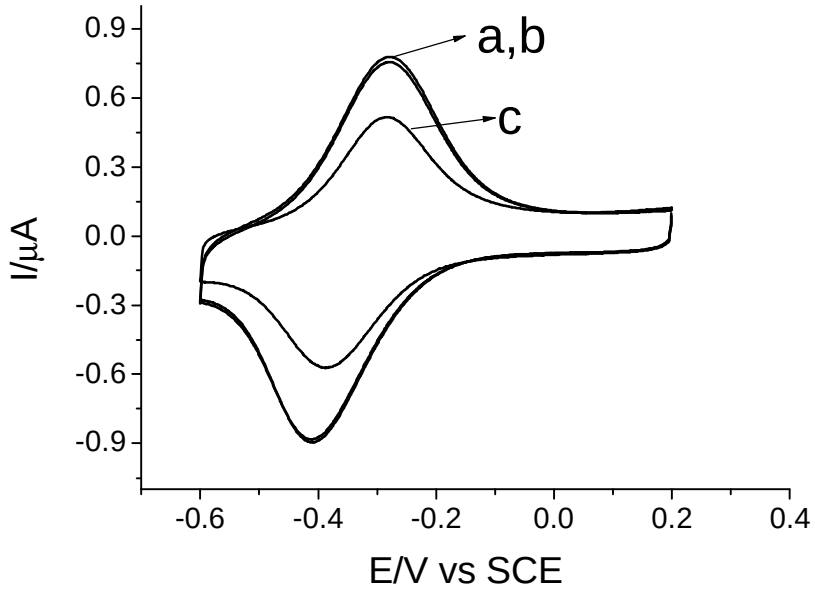

Figure S14. Cyclic voltammograms for Hb/MPPA/Au electrode in 4.4 mM PBS (pH 7.0) at a scan rate of 100 mVs^{-1}. a) without the pretreatment of potentiostatic polarization, with the pretreatment of potentiostatic polarization at b) 0.2 V and c) -0.55 V in 4.4 mM PBS (pH 7.0) for 600s.

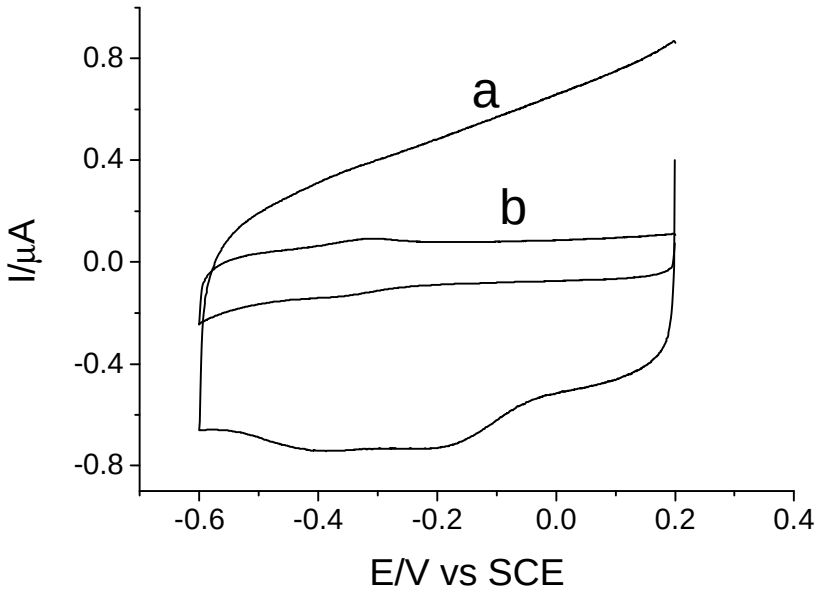

Figure S15. Cyclic voltammograms of a) bare Au and b) MPPA/Au in the 4.4 mM PBS (pH 7.0) at a scan rate of 100 mVs^{-1}.