Electronic Supplementary Information for

Probing Electron Transfer Between Hemin and Riboflavin Using A Combination of Analytical Approaches and Theoretical Calculations

Wen-Lan Wang*, Yuan Min*, Sheng-Song Yu, Wei Chen, Jie-Jie Chen**, Xiao-Yang Liu, Han-Qing Yu**

Department of Chemistry, University of Science & Technology of China, Hefei, 230026, China

*These authors contributed equally to this work.

Corresponding authors:

Dr. Jie-Jie Chen, Fax: +86 551 63601592; E-mail: chenjiej@mail.ustc.edu.cn;

Prof. Han-Qing Yu, Fax: +86 551 63607592; E-mail: hqyu@ustc.edu.cn

Table S1. Gibbs Free Energy Change (ΔG^{O}), Activation Energy (E_a), Entropy of Activation ($\Delta^{\neq}S^{O}$), and Rate Constant (k_{ET}) for the Hemin(III) to Hemx(II) Reduction with or without RF_{ox} Cofactor in Aqueous Solution at Room Temperature (298.15 K) and Atmospheric pressure.

Reaction	ΔG^{O} (kcal/mol)	E_a (kcal/mol)	$\Delta^{\neq}S^{O}$ (cal/mol·K)	k_{ET} (s^{-1})
Hemin(III) + H_3O^+ + OH^- + e^- ⇌ Hemx(II) + $2H_2O$	-127.690	0.011	-12.247	1.284×10^{10}
Hemin(III) - RF_{ox} + H_3O^+ + OH^- + e^- ⇌ Hemx(II) - RF_{ox} + $2H_2O$	-190.819	1.030	8.558	4.602×10^{14}

Table S2. Standard Gibb's Free Energy Change (ΔG^{O}) and Enthalpy Change (ΔH^{O}) of the Reactions Involved in the Electron and Proton Transfer Reaction of Porphyrin Iron and RF in Aqueous Solution at Room Temperature and Atmospheric Pressure.

Reaction	ΔG^{O} (kcal/mol)	ΔH^{O} (kcal/mol)
Hemx(II) ⇌ Heme(II)	1.067	0.039
Hemx(II) - RF_{ox} ⇌ Heme(II) - RF_{ox}	-130.414	-128.323
RF_{ox} + Heme(II) ⇌ $RF_{rad}^{\bullet -}$ + Hemin(III)	-55.077	-56.077
$RF_{rad}^{\bullet -}$ + H_3O^+ ⇌ $RF_{rad}H^{\bullet}$ + H_2O	-37.576	-38.168
$RF_{rad}^{\bullet -}$ + Heme(II) ⇌ RF_{red}^{2-} + Hemin(III)	-23.323	-25.379
$RF_{rad}H^{\bullet}$ + Heme(II) ⇌ $RF_{red}H^{-}$+ Hemin(III)	-43.924	-45.612
RF_{red}^{2-} + H_3O^+ ⇌ $RF_{red}H^{-}$ + H_2O	-58.177	-58.401
$RF_{red}H^{-}$ + H_3O^+ ⇌ $RF_{red}H_2$ + H_2O	-22.217	-22.338

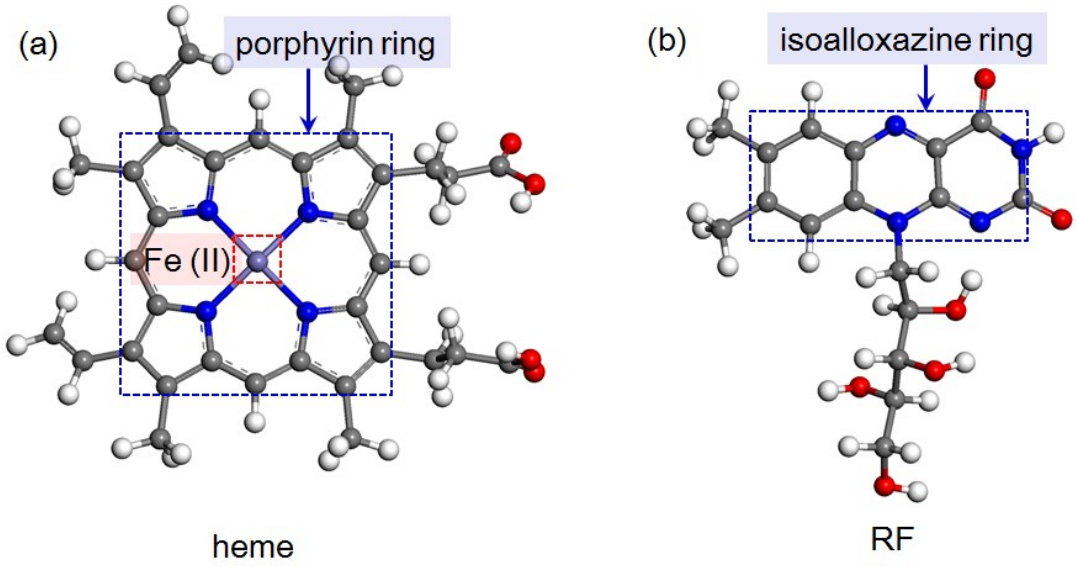

Figure S1. Energy-minimized structures of heme(II) and RF_{ox}. (a) Heme(II) structure with porphyrin ring and iron (II); and (b) RF_{ox} structure with N-heterocycle isoalloxazine ring

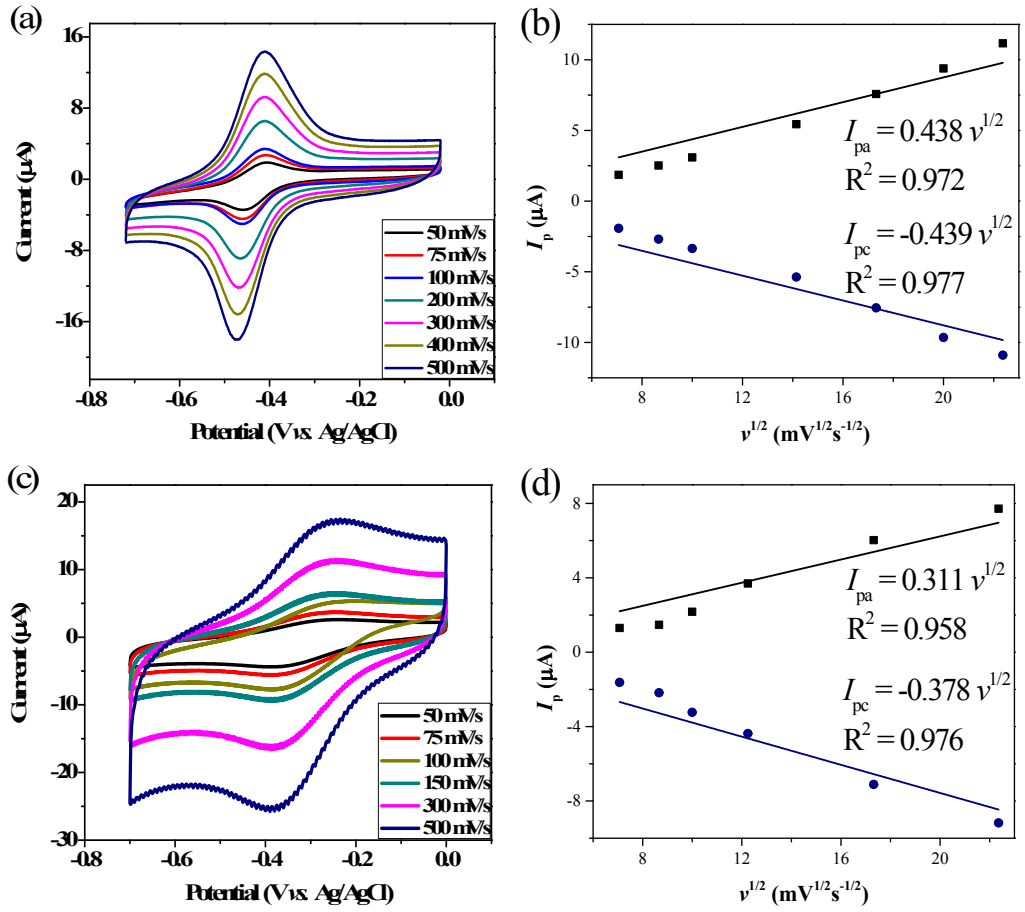

Figure S2. (a) CVs of 50 μM RF at a CPE at scan rates from 50 to 500 mV/s in the TL-cell. Scan range: 0 to -0.70 V vs. Ag/AgCl; working electrode: CPE with a surface area of 0.6 cm^2; buffer solution: PBS (pH = 7.0); (b) Relationship between RF peak current (the first step of reduction and oxidation) and scan rate; (c) CVs of 50 μM Hemin(III) at a CPE at scan rate from 50 to 500 mV/s in the TL-cell. Scan range: 0 to -0.70 V vs. Ag/AgCl; working electrode: CPE with a surface area of 0.6 cm^2; buffer solution: PBS (pH = 7.0); and (d) Relationship between Hemin(III) peak current and scan rate

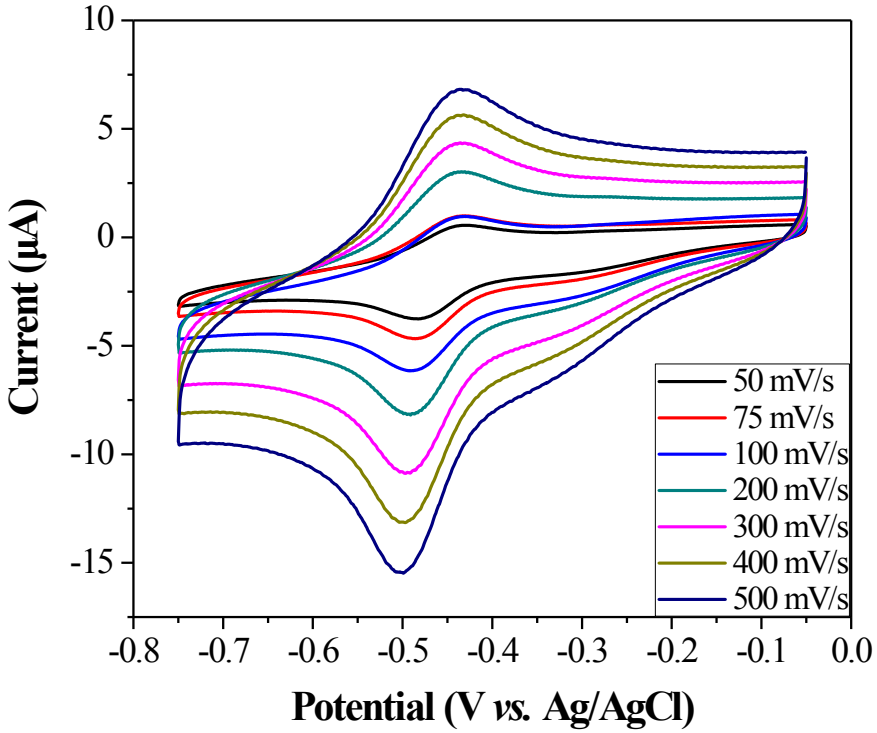

Figure S3. CV of 50 μM RF and 50 μM Hemin(III) mixture in the TL-cell at a scan rate from 50 to 500 mV/s. Scan range: -0.05 to -0.75 V vs. Ag/AgCl; buffer solution: PBS (pH = 7.0); working electrode: CPE with a surface area of 0.6 cm^2

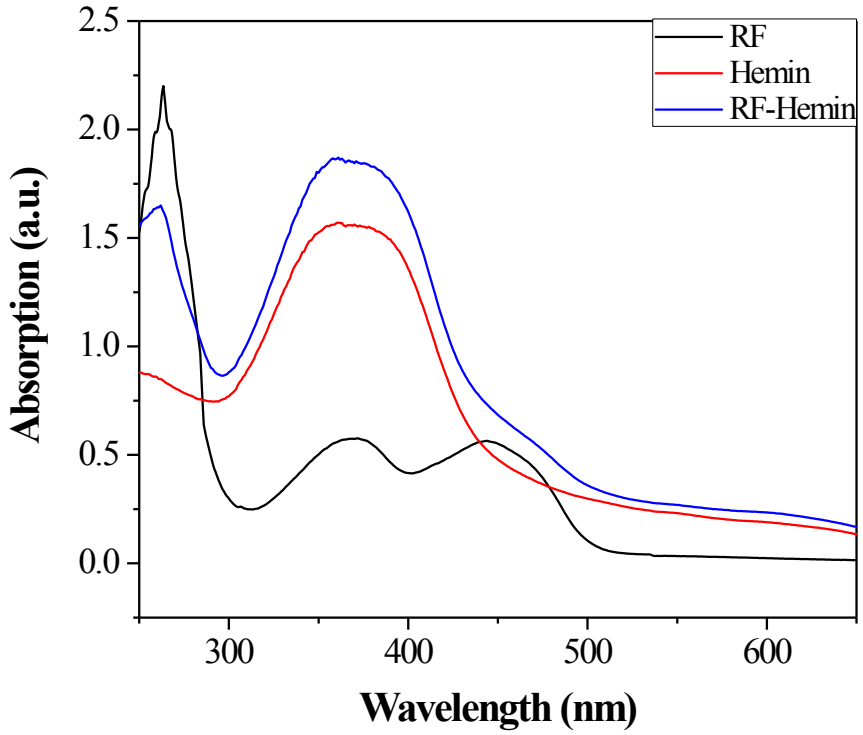

Figure S4. UV-Vis spectrum of 50 μM RF, 50μM hemin(III) and 50 μM RF-hemin(III) mixture in the TL-cell without electron supply. Buffer solution: PBS (pH = 7.0)

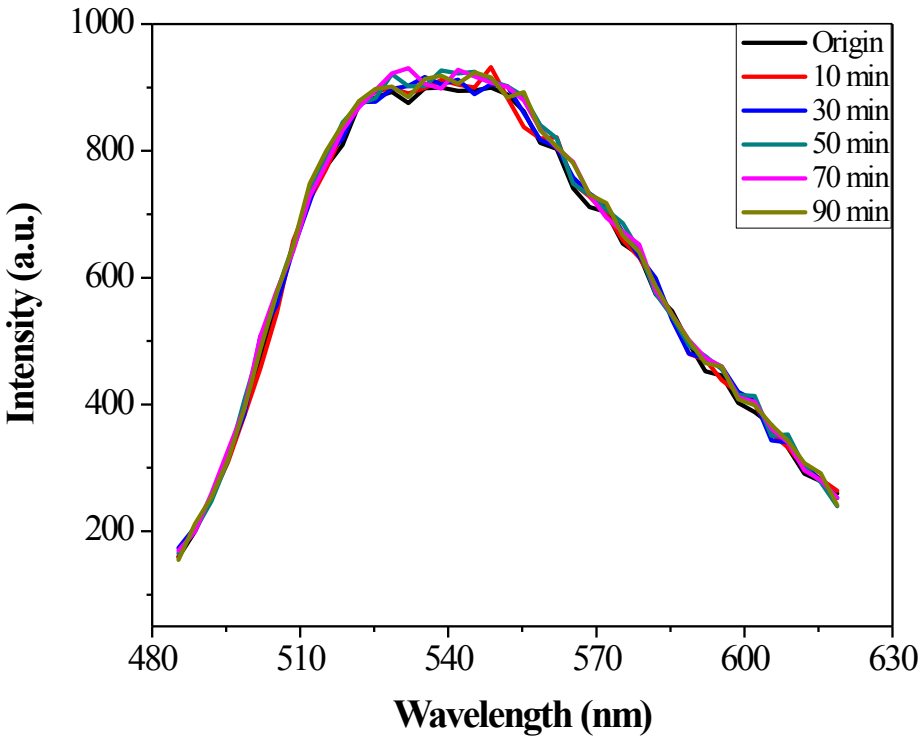

Figure S5. *In situ* electrochemical fluorescence spectra of RF at -0.415 V *vs.* Ag/AgCl for 90 min in the TL-cell. Excitation wavelength was 444 nm; the excitation and emission slits were both 5 nm; scanning speed was set at 600 nm/min, wavelength of 490-620 nm; buffer solution: PBS (pH = 7.0)