Supporting Information

Structure orientation of hemin self-assembly layer determining the direct electron transfer reaction

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Experimental section

Materials:

Hemin (Sigma), L-Histidine monohydrochloride monohydrate (His, Sigma), 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES, Sigma), 6-Amino-1-hexanethiol hydrochloride (MHN, Dojindo Laboratories, Japan), N-Hydroxy-succinimide (NHS, Aldrich), Octadecanethiol (98%, ODT, Aldrich), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, Alfa Aesar) were of reagent grade quality or better and used without further purification. Hydrogen peroxide (H_2O_2, 30%) was of analytical grade. Aqueous solutions were prepared from deionized water (>18 MΩ·cm, Milli-Q purification system).

Apparatus and procedures

Electrochemical measurements were carried out using a CHI 660D electrochemical workstation (CH Instruments) in a three-electrode cell with an Ag/AgCl (3 M KCl) solution as the reference and a Pt wire as the counter electrode. Buffers were purged with high-purity nitrogen for at least 10 min prior to electrochemical measurements, and a nitrogen environment was then maintained over the electrolytes during measurements. All the measurements were performed under ambient conditions (20±2°C). XPS analyses were carried out on a Thermo Fisher X-ray photoelectron spectrometer system equipped with Al radiation as a probe, with a chamber pressure of 5×10^{-9} Torr. Power of the X-ray source was kept constant at 150 W.

Before assessment of reactive radical, 2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA) was initially dissolved in a small amount of ethanol and then hydrolyzed by NaOH solution into 2′,7′-Dichlorodihydrofluorescein (DCFH). The resultant DCFH can be oxidized by reactive radical or intermediate to fluorescent 2′,7′-Dichlorofluorescein (DCF) which is used as an indicator of the production of reactive radical during the catalytic reduction process of H_2O_2 by hemin. Electrochemical method with a CHI 660D electrochemical workstation combined with fluorescence spectrometer (Varian Cary Eclipse, USA) was used to assess the reactive radical. In the presence of DCFH, the H_2O_2 is reduced catalytically by hemin at a certain potential, simultaneously, the fluorescence spectrum of the resulting reaction solution is taken. According to the appearance or absence of characteristic peak of DCF, we can determine whether there are reactive radical formed during the catalytic reduction process of H_2O_2.

A gold disk electrode (diameter 2.0 mm) was polished with 1.0, 0.3, 0.05 μm alumina slurry sequentially, and was then electrochemically cleaned in a 0.5 M H_2SO_4 solution between 0 V and 1.5 V at a scan rate of 0.5 $V\cdot s^{-1}$ until reproducible voltammograms were obtained. The gold nanofilm was prepared according to the previous work.[1] The clean electrode was anodized at 5 V in 0.1 M phosphate buffer solution (pH 7.4) for 3 min to form oxidized gold surface. Then, the film of gold oxides was chemically

reduced in a 1.0 M β-D-glucose aqueous solution at room temperature. This process resulted in a change of the electrode color from salmon pink to black, indicating the formation of porous gold nanofilm. Real surface area of the bare gold disk electrode was determined from the cyclic voltammogram by integration of the cathodic peak for the reduction of surface gold oxide in a 0.5 M H_2SO_4 solution.[2,3] The roughness factor was calculated as the ratio of real surface area to geometric area to be about 17.

In situ surface-enhanced infrared absorption spectroscopy (SEIRAS) was performed on a Bruker Tensor 27 (Bruker, Germany) equipped with a liquid-nitrogen-cooled mercury cadmium telluride (MCT) detector. A thin gold film with thickness of ca. 100 nm was firstly formed on the flat surface of a half-cylindric ZnSe prism by chemical deposition. Then, a reference spectrum of 6-Amino-1-hexanethiol hydrochloride (MHN) SAM-modified gold nanofilm in 25 mM HEPES (pH 7.0) was recorded in the absence of hemin. Subsequently, 10 μM hemin containing 1 $mg \cdot mL^{-1}$ EDC and 1 $mg \cdot mL^{-1}$ NHS were added to the cell and the final concentration of hemin was 5 μM. The sample spectra were collected in the wavenumber range between 1000 and 4000 cm^{-1} over 128 scans at a resolution of 4 cm^{-1}.

Construction of experimental models

Hemin-His model: NH_2-terminated 6-Amino-1-hexanethiol hydrochloride (MHN) was first self-assembled on a prepared Au nanofilm electrode by immersing the porous gold electrode in aqueous solution containing 2 mM MHN for approximately 20 h, followed by rinsing with deionized water to remove the physically adsorbed thiol molecules. Then, His was covalently assembled on the MHN surface by EDC/NHS. Finally, the resulting His-MHN modified Au nanofilm electrode was immersed in 25 mM pH 7.0 HEPES solution containing 10 μM hemin. Hemin was immobilized by the association of the pyridinic nitrogen of the imidazole moiety of His with the iron porphyrin of hemin.

Hemin-MHN model with ODT: The MHN modified Au nanofilm was first fabricated by the same procedure as mentioned above. Then, hemin was covalently attached to the MHN surface by immersing the MHN modified Au electrode in 25 mM pH 7.0 HEPES solution containing 10 μM hemin, 0.5 mM EDC and 0.5 mM NHS for 20 min. The Hemin-MHN model modified Au electrode was then formed after rinsed with blank HEPES buffer and dried by N_2 flow. Octadecanethiol (ODT) is a thiol and can be attached onto the gold electrode surface via Au-S bond. Its length is almost the same as that of the Hemin-MHN model, so ODT can be employed to seal the unoccupied site on the gold electrode surface. The aim of ODT filling is to decrease the mobility of assembled hemin molecules and make them more vertical to the electrode surface. Last, the Hemin-MHN modified electrode was immersed in 2 mM alcohol solution of ODT for 30 min, followed by rinsing with absolute ethanol and deionized water respectively and dried by N_2 flow. The resulting electrode was denoted as Hemin-MHN model with ODT.

Supplementary results:

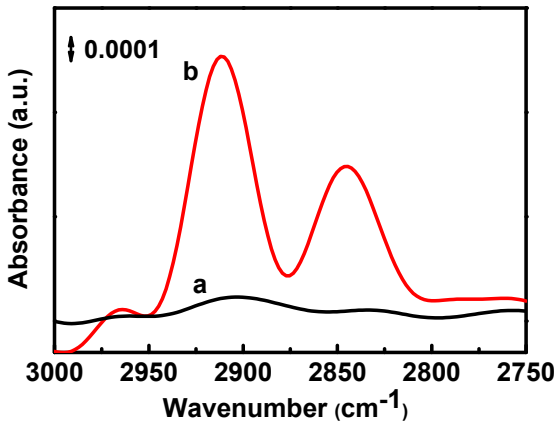

Figure S1. SEIRAS spectra of: Hemin-MHN Model in high-wavenumber region before (a) and after (b) ODT attachment.

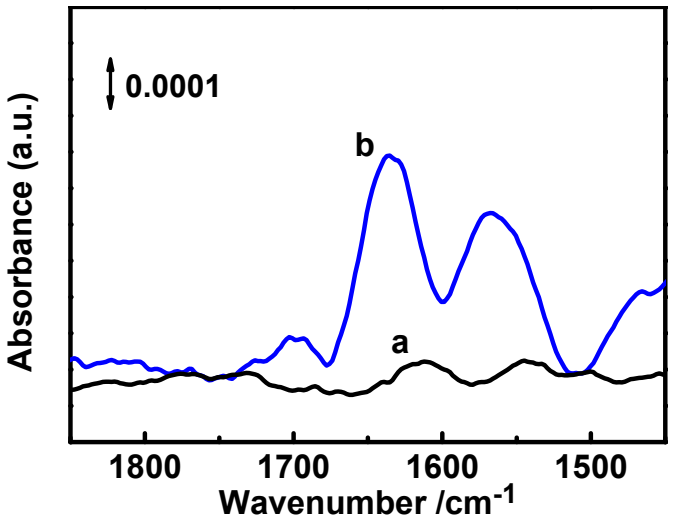

Figure S2. SEIRAS spectra of: MHN SAM before (a) and after (b) His assembling. The spectrum of MHN SAM is taken as baseline as shown in curve *a*. Two distinct bands at 1635 cm^{-1} and 1567 cm^{-1} in curve *b* are characteristics of amide I band and amide II band of the amide bond formed between His and MHN molecules respectively, which clearly indicate the attachment of His molecules on the surface.

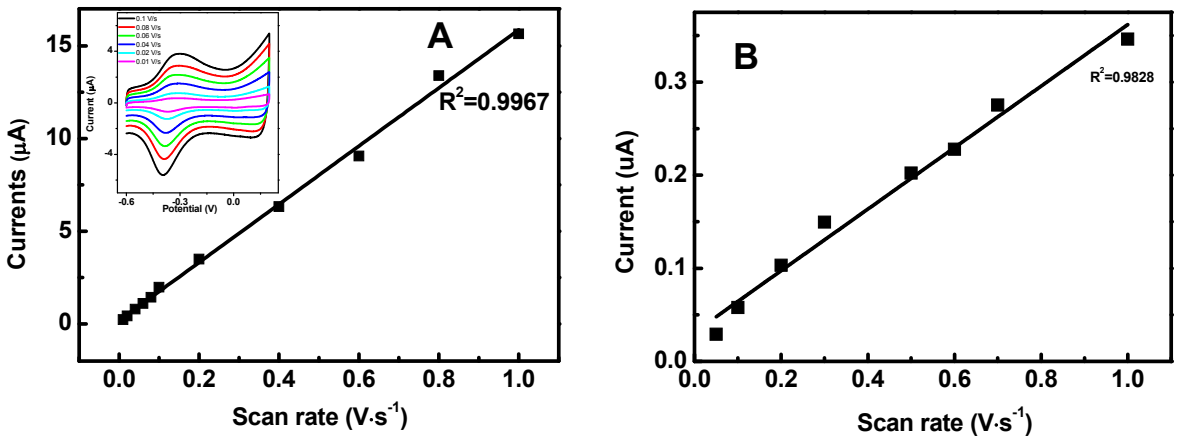

Figure S3. Plots of the anodic peak currents of hemin vs. scan rate in 25 mM pH 7.0 HEPES: (A) Hemin-His model and (B) Hemin-MHN model.

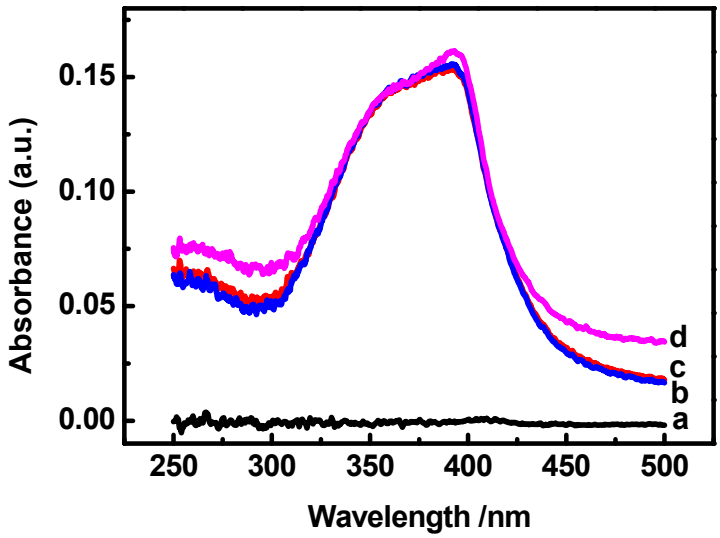

Figure S4. UV spectra of His (a) 2 μM Hemin:His 1:2 (mole ratio), (c) 2 μM Hemin:His 1:1(mole ratio) and (d) 2 μM Hemin.

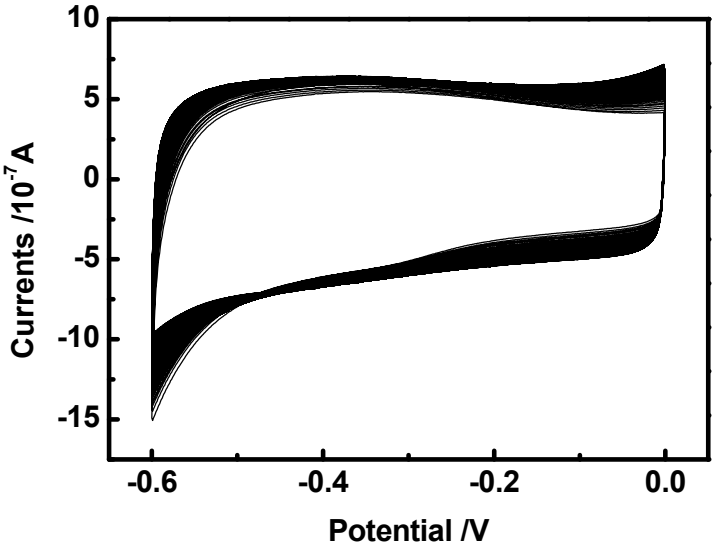

Figure S5. Cyclic voltammograms (CV) of Hemin-MHN model with ODT in 25 mM pH 7.0 HEPES containing 1 mM histidine. The scan rate was 50 $mV \cdot s^{-1}$.

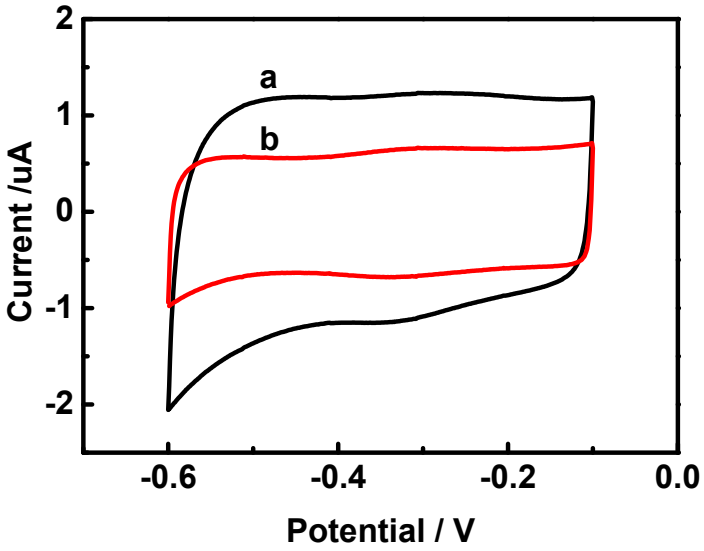

Figure S6. CVs of hemin in the Hemin-MHN model (a) without and (b) with 1-hexanethiol in 25 mM pH 7.0 HEPES. The scan rate was 50 $mV \cdot s^{-1}$

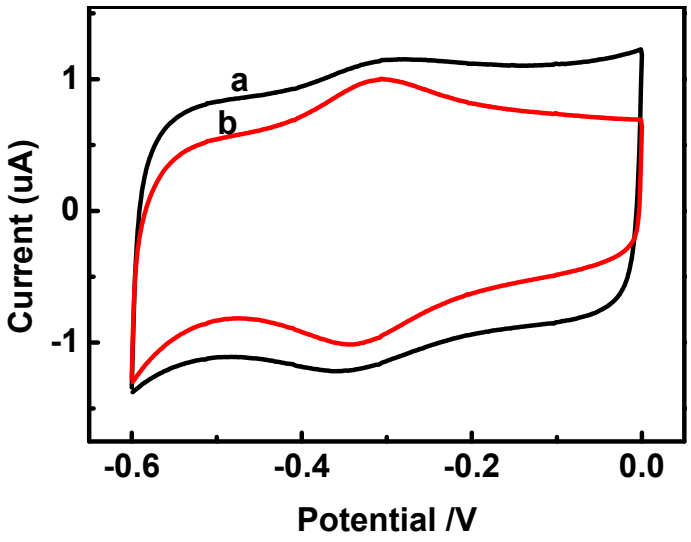

Figure S7. CVs of hemin in the Hemin-His model (a) without and (b) with ODT in 25 mM pH 7.0 HEPES. The scan rate was 50 $mV \cdot s^{-1}$

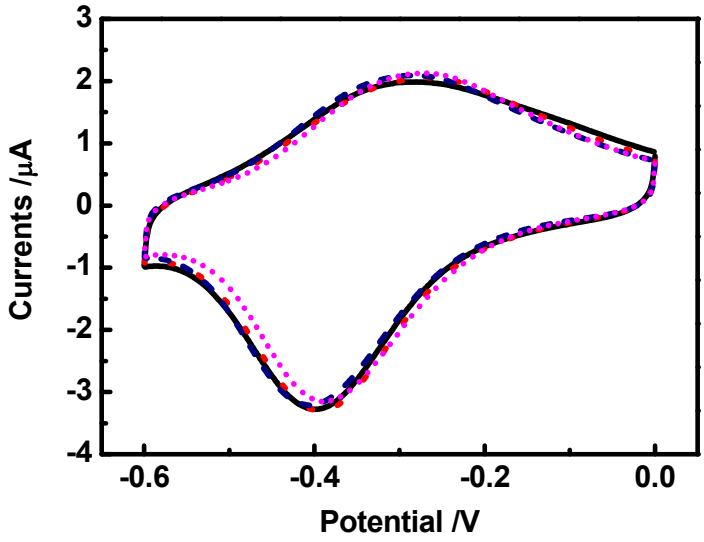

Figure S8. CVs of hemin in the Hemin-His model in 25 mM HEPES (pH 7.0) in the absence of EDTA (solid curve), in the presence of 1 mM EDTA for 10 min (dotted curve), 10 h (short dashed curve) and 5 mM EDTA for 10 h (short dotted curve). The concentration of hemin used in (B) is 50 μM.

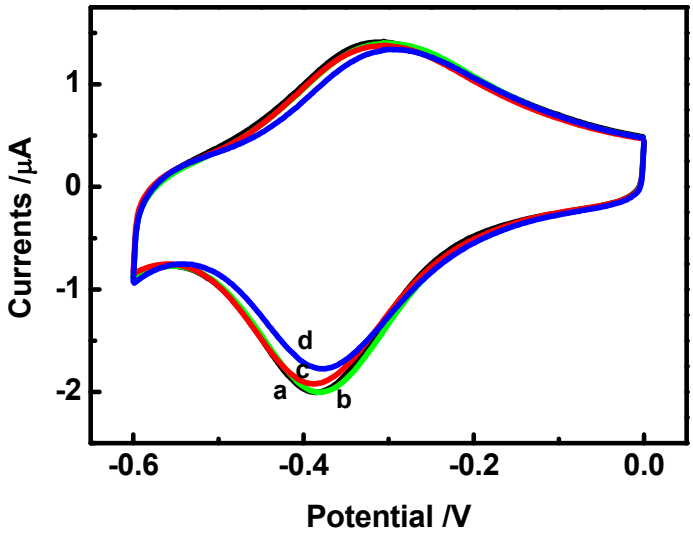

Figure S9. CVs of hemin in the Hemin-His model in 25 mM HEPES (pH 7.0) in the absence of His (a), in the presence of 20 μM EDTA for 10 min (b), 10 h (c) and 3 mM His for 3 h (d).

Supplementary references

1. G. X. Wang, W. J. Bao, M. Wang, X. H. Xia, *Chem. Commun.*, 2012, **48**, 10859.
2. L. Su, F. Gao and L. Mao, *Anal. Chem.*, 2006, **78**, 2651.
3. J. M. Abad, M. Velez, C. Santamaria, J. M. Guisan, P. R. Matheus, L. Vazquez, I. Gazaryan, L. Gorton, T. Gibson and V. M. Fernandez, *J. Am. Chem. Soc.*, 2002, **124**, 12845.