Supporting Information

Heme plane orientation dependent direct electron transfer of cytochrome *c* at SAMs/Au electrodes with different wettability

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Experimental section

Materials:

6-Mercapto-1-hexanol (MH), 1-hexanethiol (HT) and horse heart cytochrome *c* were purchased from Sigma-Aldrich and used as received without any further purification. Phosphate buffer solutions (PBS, pH 7.0) were prepared with KH_2PO_4 and K_2HPO_4. In all experiments, deionized water (>18 MΩ cm) from a Milli-Q purification system (Purelab Classic Corp., USA) was used. All chemicals were of reagent grade.

Apparatus and procedures

Cyclic voltammetry (CV) was performed with a CHI 660D electrochemical workstation (CH Instruments) in a three-electrode cell with an Ag/AgCl electrode in 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 experiments. All measurements were performed under ambient conditions.

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 silicon prism by chemical deposition. Then, a reference spectrum of a thiol-modified gold nanofilm in 10 mM phosphate buffer (pH 7.0) was recorded in the absence of cyt *c*. Subsequently, cyt *c* dissolved in the same buffer solution was added to the cell to achieve a final concentration of 1 mg/mL^1. 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}.

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. No X-ray induced decomposition of samples as manifested by changing peak areas or positions was observed during XPS data acquisition. Samples were analyzed immediately following withdrawal from the ethanol/thiol or cyt *c* solutions, rinsing with ethanol and DI water respectively, and transport through air to a load-lock chamber for introduction into the surface analysis system. The analysis spot size was 400 μm in diameter. The binding energy was calibrated by means of the Au 4f7/2 peak energy of 84.0 eV. Detailed S 2p, P 2p, N 1s, and Fe 2p signals in immobilized cyt *c*

were also collected and analyzed. For each modified film, the relative content of N in adsorbed cyt *c* vs. Au substrate can be calculated from the XPS results, and can be used to quantitatively obtain the amount of immobilized cyt *c*. The ratio (denoted as *f*) of the N relative content of cyt *c* on one film surface to that on MH film is taken as the criterion to compare the immobilization amount. Thus, *f* representing the immobilized amount of cyt *c* on MH surface with saturation adsorption time of 6 h is normalized as 1.0.

The wettability of the sample surfaces was determined using sessile drop techniques. A drop shape analysis system (Easy Drop DSA20E, Kruss, Germany) with analysis software (DSA1 version 1.80, Kruss, Germany) was used to measure the contact angles (θ_{CA}).

Assembly of cyt *c*/thiol-SAMs/p-Au electrode

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. Gold nanofilm was prepared according to the previous work. The clean electrode was first anodized at 5 V in 0.1 M phosphate buffer solution (pH 7.4) for 5 min to form oxidized gold surface, and the surface color changed from yellow to salmon pink. Then, the gold oxides were chemically reduced in a 1.0 $mol \cdot L^{-1}$ β-D-glucose aqueous solution at 4 °C for 5 min. This process resulted in change of the electrode color from salmon pink to black, indicating the formation of a 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.[1,2] The roughness factor was calculated as the ratio of real surface area to geometric area to be about 21.

Five different types of thiol-SAMs, which have contrasting chemistries by varying stoichiometric ratios of MH to HT ligands, were formed on the prepared nanoporous Au film electrodes by immersing in single thiol or mixed thiol ethanolic solution with the total concentration of 4.5 nmol for approximately 20 h. Thus, the composition of the corresponding SAMs were: 100% MH, 2:1 MH:HT (67% MH), 1:1 MH:HT (50%), 1:2 MH:HT (33%), and 100% HT.[3] Then, the resulting electrodes were thoroughly rinsed with ethanol and water to remove the physically adsorbed thiol molecules. For cyt *c* adsorption, the SAMs modified electrodes were incubated in a 10 mM pH 7.0 PBS solution containing 1 $mg \cdot mL^{-1}$ of cyt *c* at 4 °C for 6 h. Subsequently, the formed cyt *c*/thiol-SAMs/AuNp electrodes were rinsed by bulk PBS thoroughly to remove any loosely bounded cyt *c* from the electrode surfaces.

The same procedure was also used to fabricate the samples for X-ray photoelectron spectroscopy (XPS, KR) characterization, contact angles measurement and SEIRA-ATR measurement, although the substrates were different.

Supplementary results:

Table S1. The contact angles for different thiol-SAMs-porous Au surfaces after cyt *c* adsorption.

Films	MH	2:1 MH:HT	1:1 MH:HT	1:2 MH:HT	HT
θ_{CA}	20.0±0.9°	30.3±0.3°	38.8±0.5°	54.7±0.4°	117.3±0.7°

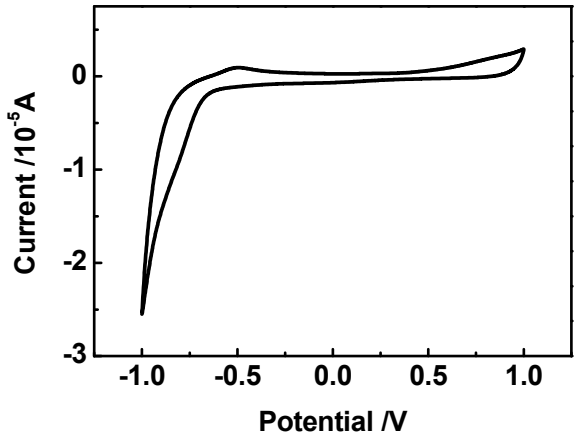

Figure S1. Cyclic voltammogram of cyt *c* adsorbed to HT-SAMs modified Au electrode when broadening the potential scan range from -1.0 to 1.0 V in 10 mM PBS (pH 7.0) at a scan rate of 100 mV·s^{-1}.

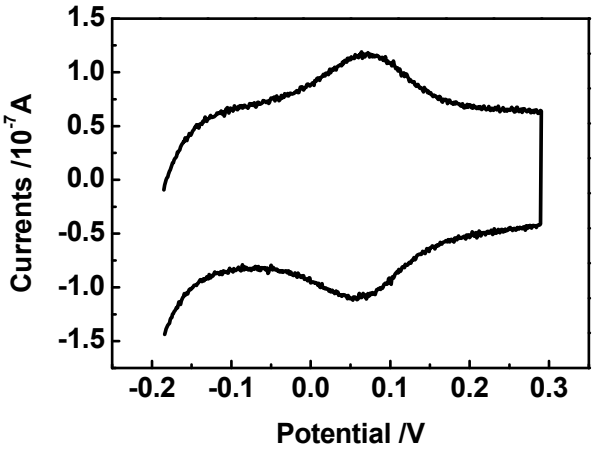

Figure S2. Cyclic voltammogram (background was subtracted) of cyt *c* adsorbed on the MH-SAMs modified Au electrode with 20 min of the adsorption time of cyt *c* in 10 mM PBS (pH 7.0) at a scan rate of 100 mV·s^{-1}.

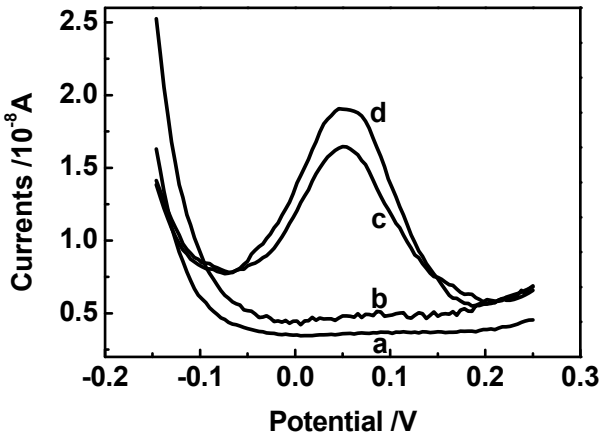

Figure S3. Differential pulse voltammograms of cyt *c* adsorbed on SAMs/Au electrode with different hydrocarbon chain length in 10 mM PBS (pH 7.0) at a scan rate of 100 mV·s^{-1}: a) HT-SAMs and b) PT-SAMs with saturated adsorption of cyt *c*, c) and d) MU-SAMs with 1h and 2h of cyt *c* adsorption time respectively. The corresponding *f* values for immobilized cyt *c* are 0.2286 (curve c) and 0.4386 (curve d), respectively.

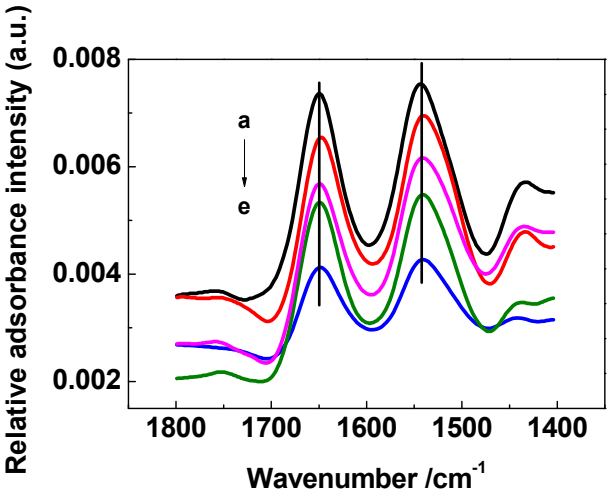

Figure S4. SEIRA spectra of cyt *c* saturation adsorbed on different thiol-SAMs surfaces under pH 7.0 solutions. From the top down, MH (a), 2:1 MH:HT (b), 1:1 MH:HT (c), 1:2 MH:HT (d), HT (e) films. The displayed spectra were taken at 1 h after addition of cyt *c*. The reference spectrum is taken in 10 mM pH 7.0 PBS without cyt *c*.

Supplementary references

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2. 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*, 12845-12853.
3. A. Stewart, S. Zheng, M. R. McCourt and S. E. J. Bell, *ACS Nano* **2012**, *6*, 3718.