Supplementary Information

Strong Electron-conjugation Interaction Facilitates Electron Transfer of Hemoglobin by Ce(OH)_3 Nanorods

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Materials and methods

Synthesis of NRs: Ce(OH)_3 NRs were synthesized according to our previous methods with minor modifications. Typically, 1.5 g CeCl_3·7H_2O was dissolved in distilled water and 4 g NaOH was rapidly added with vigorous stirring. After 10 minutes of stirring, all of the mixture were then transferred into a Teflon-lined, stainless autoclave at 130 °C for 18 h. The products were collected by filtration, washed with distilled water to remove any possible ionic remnants, and then dried at room temperature for 20 h. The preparation of CeO_2 NRs followed the same procedure except one additional step-calcedine at 200 °C for 6 h.

Preparation of Hb/Ce(OH)_3 /CHT/GCE: Stock solution of 10 mg ml^{-1} Hb (from sigma) was prepared by directly dissolving Hb in 0.05 mol L^{-1} phosphate buffer solution (PBS, pH 7.0) and stored at 4 °C. CHT (from crab shells, minimum 85% deacetylated) solution (1.0 mg ml^{-1}) was prepared by dissolving 1 mg CHT in 1 mL 0.05 mol L^{-1} acetic acid. The Ce(OH)_3 NRs suspension (2 mg ml^{-1}) was prepared by dispersing Ce(OH)_3 NRs in deionized water with stirring overnight. To obtain the best CV
responses, the ratio of Hb/Ce(OH)$_3$/CHT has been optimized. Finally, the mixing solution of 2 mg ml$^{-1}$ Hb, 0.8 mg ml$^{-1}$ Ce(OH)$_3$ and 0.4 mg ml$^{-1}$ CHT was used to prepare the Hb/Ce(OH)$_3$ NRs/CHT/GCE. Before each experiment, glassy carbon electrode (GCE) was polished with 1, 0.3, 0.05 µm alumina powder on a polishing cloth. Then the electrode was successively sonicated in ethanol and double distilled water. After the electrode dried by N$_2$, 4.5 µl Hb-Ce(OH)$_3$-CHT dispersion was dropped on the pretreated GCE surface. The electrode was left in a glass bottle in air until dry, thus the Hb/Ce(OH)$_3$/CHT/GCE was obtained. The Hb/CeO$_2$/CHT/GCE was made following the same procedures.

**Apparatus and measurements:** Electrochemical experiments were performed on a CHI 440 electrochemical station (CH Instruments, China) with a conventional three-electrode system. The glass carbon electrode with modified film acted as the working electrode. The Ag/AgCl (saturated KCl) was used as the reference electrode and the Pt wire acted as the counter electrode. Phosphate buffer solutions (PBS, 0.05 M, pH 8.5) were used as the supporting electrolyte. Prior to a series of electrochemical experiments, buffers were purged with highly purified nitrogen for at least 40 min and N$_2$ environment was kept in the whole experiment process.

**Characterization of the materials:** Powder X-Ray Diffraction (XRD) was performed with monochromatized Cu Kα radiation (λ = 1.5418 Å). FTIR spectra were collected on Avatar 360 FTIR. Samples films were collected on BaF$_2$ sheet for IR testing. The size and morphology of all the NRs were obtained with a Tecnai G$^2$20S-Twin transmission electron microscope (TEM). The synthesized NRs were dispersed in the ethanol to prepare the TEM samples. X-ray photoelectron spectroscopy (XPS) analysis was performed on an ESCALAB 250Xi (Thermo Scientific) with Al Kα radiation in twin anode at 14 kV ×16 Ma, calibrated internally by carbon deposit C (1s) binding energy (BE) at 284.6 eV.
Electron Paramagnetic Resonance (EPR) measurements were carried out at 77 K with a JES-FA200 spectrometer operating in the X-band (9.0 GHz). The microwave power was 0.998 mW and other acquisition conditions, such as modulation amplitude, time constant, were adjusted to achieve optimal signal-to-noise ratio without signal distortion or saturation. All EPR samples were frozen by immersing in liquid nitrogen and then placed in the spectrometer rectangular cavity. Samples were prepared by dispersing 20 mg ml⁻¹ Ce(OH)₃/CeO₂ NRs and 5 mg ml⁻¹ hemoglobin in 0.05 M PBS (pH 8.5). The mixed system was oscillated for approximately 1 h and certain amount of the dispersing solution was taken out for the EPR measurements.

**XPS analysis on both NRs**

X-ray photoelectron (XPS) analysis was carried out to characterize the valence state of Ce ions. The Ce 3d level has a very complicated structure. **Fig.S1** shows the Ce 3d XPS spectra of both Ce(OH)₃ and CeO₂ NRs. Six peaks labeled as V, V'', V''' (3d₅/₂), U, U', U''' (3d₃/₂) referring to three pairs of spin-orbit doublets can be identified from CeO₂ NRs and they are characteristic of Ce⁴⁺ 3d final states. The Ce 3d spectrum from Ce(OH)₃ NRs shows two pairs of doublets [noted as U₀, U', V₀, V'] corresponding to Ce³⁺ 3d states.³ ⁴ It is worth noting that U''' peak corresponding to Ce⁴⁺ 3d state is also observed on Ce(OH)₃ samples, only with lower intensity than those peaks belong to Ce³⁺. This is attributed to the strong reducing property of Ce³⁺ ions, which lead to partial oxidation of surface Ce³⁺ on Ce(OH)₃ NRs.
HRTEM analysis on the NRs

Eliminating the influence of hydroxyls

Many factors like electrostatic interactions, hydrogen bonding, and hydrophobic effects between proteins and nanostructures have been proposed to affect the electron-transfer process between proteins and the electrode. In the present study, the discrepancy between two NRs modified Hb lies in their distinct compound type: hydroxide versus oxide. As well documented by the literatures, abundant surface hydroxyls could impact on the electrocommunication between redox proteins and the electrode,
hence Hb modified with Y(OH)$_3$ and Y$_2$O$_3$ NRs were chosen to conduct cyclic voltammograms tests given the similarities between these two couples: both elements are lanthanide and they are of the same type. The only difference between the two couples lies in the fact that Y(OH)$_3$ NRs do not possess reducing properties compared with Ce(OH)$_3$ NRs.

From Fig. S3 we can see that both Y(OH)$_3$ and Y$_2$O$_3$ NRs could realize the electrochemical redox reaction between Hb-Fe(III) and Hb-Fe(II). However, the redox potentials for both films especially the reduction potentials didn’t engender any difference. This indicates that surface hydroxyls are not of vital importance in the current system. Based on the above, we believe that, for other lanthanide hydroxide and oxide, there would be no interaction difference between the two nano-bio systems as long as the lanthanide element in the compounds has the same valent state.

![Cyclic voltammograms](https://example.com/cyclicvoltammograms.png)

**Figure S3.** Cyclic voltammograms of Hb/Y$_2$O$_3$/CHT/GCE (black curve) and Hb/Y(OH)$_3$/CHT/GCE (red curve) and in 0.05M PBS solution (pH 8.5) at scan rate 100mV/s.

**EPR Spectra of CeO$_2$ NRs with aromatic amino acids**

**Fig. S4** describes the EPR spectra of CeO$_2$ NRs interacting with Histidine. It is easy to find that CeO$_2$ NRs are incapable of interacting with Histidine since the EPR signals of CeO$_2$/Histidine are comprised of peaks from nanoceria.
**Figure S4** EPR spectra of (a) 5mg/ml Histidine; (b) 20mg/ml CeO₂ NRs; (c) 20mg/ml CeO₂ NRs and 5mg/ml Histidine in 0.05M PBS (pH 8.5) at 77 K.

**References**