Electronic Supplementary Material (ESI) for Chemical Communications


# An efficient visible light controlled protein delivery system

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## Experimental:

### Sample preparation:

**Synthesis of $TiO_2$-DB:** 0.012g of 3,4-dihydroxy benzoic acid (Sinopharm Chemical Reagent Co. Ltd)was dissolved in 200mL pH 2 hydrochloric acid aqueous solution. Then 1.0g P25 (Degussa) was suspended in the solution. After sonic dispersion for 5min, the mixture was stirred at 60℃ for 5h. The product was separated by centrifugation at 8000rpm for 15min and wash with distilled water once. Finally, the resulting material was dried in vacuum. By measuring the absorbance of DB in the solution before and after adding $TiO_2$, it was found 7.8mg of DB was absorbed.

In order to ensure the structure of 3,4-dihydroxyl benzoic acid, benzoic acid, 4-hydroxyl benzoic

acid, 3,4-diamino benzoic acid, 2,3-dihydroxyl benzoic acid and 1,2,4,5-benzenetetracarboxylic acid were also dissolved in pH 2 hydrochloric acid aqueous solution with the same concentration as 3,4-dihydroxyl benzoic acid. Then 1.0g P25 was suspended in these solutions and stirred for 5h at 60℃. The respective samples were collected though the same steps.

**Synthesis of $TiO_2$-DB-Hb:** $TiO_2$-DB-Hb was synthesis by the reaction between carboxyl group of $TiO_2$-DB and amine of Hb using 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide (EDC) (Aladdin Reagent Co. Ltd ) and N-hydroxy-2,5-pyrrolidinedione (NHS) (Aladdin Reagent Co. Ltd) to activate the carboxyl group. Typical, 0.4g of $TiO_2$-DB was dispersed in 200mL pH 5.44 phosphate buffer solution (PBS). Then 0.2g of EDC 0.2g of NHS was dissolved in the suspension, respectively. After shaking at 350rpm for 20min, the pH of the mixture was adjust to 7.2 by adding $Na_2HPO_4 \cdot 12H_2O$. 0.06g of hemoglobin (Hb) (Solarbio Science & Technology Co. Ltd) was added after $Na_2HPO_4 \cdot 12H_2O$ was dissolved. The suspension was shaken for 5h at 350rpm. 0.055g Hb was linked to $TiO_2$-DB after reaction. The final hybrid material was separated by centrifugation at 6000rpm for 10min. After wash with distilled water for three times, the sample was dried in vacuum.

## Hb delivery:

**Hb delivery under visible light irradiation:** Hb delivery experiment was carried out at a Preyx reactor with a water jacket. 0.4g of $TiO_2$-DB-Hb was dispersed in 100mL pH 6.7 PBS at 25℃. The light resource contains a 300W Xe lamp with a filter (λ>420nm) to ascertain the irradiate light was visible light. 5mL suspension was extracted at different interval and centrifugated at 6000rpm for 5min to separate the solid from the solution.

**Irradiate $TiO_2$-DB with visible light (λ>420nm):** The experiment of $TiO_2$-DB irradiation with visible light (λ>420nm) is the same as the Hb delivery experiment. 0.4g of $TiO_2$-DB was dispersed in 100mL pH 6.7 PBS. Then the suspension was irradiated with visible light (λ>420nm) for 10h. The irradiated sample was separated by centrifugation at 8000rpm for 15min and dried in vacuum for XPS analysis.

## Activity of the released Hb:

**Catalysis activity of the released Hb:** The released Hb was used to catalyze the reaction between 1,2-diaminobenzene and hydrogen peroxide. The reaction was performed in 25mL pH=6.7 PBS. 7.4mmol/L of 1,2-diaminobenzene (Nanjing Chemical Reagent Co. Ltd) and 0.12mol/L of hydrogen peroxide (Nanjing Chemical Reagent Co. Ltd) were used as the substrates and 5mL supernatant of the final suspension at 8h was added to catalyze the reaction. The reaction was monitored by measuring the absorbance of the product at 425nm along with the increasing time. For comparison, the reaction between the same concentration of 1,2-diaminobenzene and hydrogen peroxide without adding released Hb was also carried out.

# Characterization:

Uv-vis diffuse reflection spectra of the samples were performed using a Shimaduzu UV-2550

Spectrophotometer and converted from reflection to absorbance by the Kubelka-Munk method. Uv-vis absorbance spectra were carried out at Shimaduzu Uv-1750 spectrophotometer. Infrared spectra (IR) were taken in fourior transform mode using a Nicolet NEXUS870 FTIR spectrometer. X-ray Photoelectron Spectra were measured on a PHI-5000 Versaprobe with Al Kα radiation with the energy of 15kV 1.6mA, binding energies were calibrated versus the carbon signal at 284.6eV.

# Computation details:

All calculations are performed with Gaussian 03 program.

B3LYP/6-31g(d,p)[2]method was used to optimize $Ti_{10}O_{20}$ cluster, DB model and $TiO_2$-DB model.HOMO and LUMO orbitals of the conresponding model were constructed with Gview programbased the B3LYP/6-31g(d,p)-optimized results, respectively.

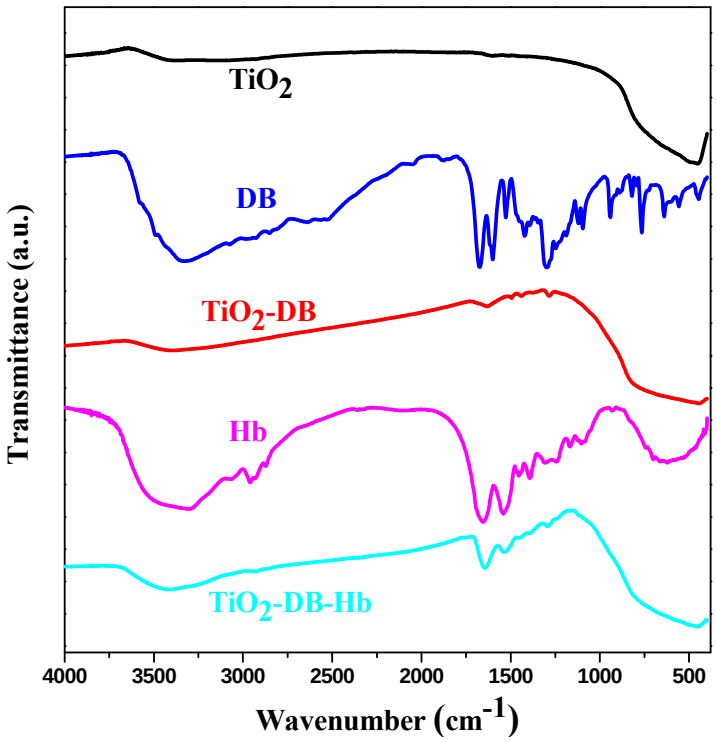


Fig.S1 FTIR spectra of $TiO_2$, $TiO_2$-DB, DB, Hb and $TiO_2$-DB-Hb

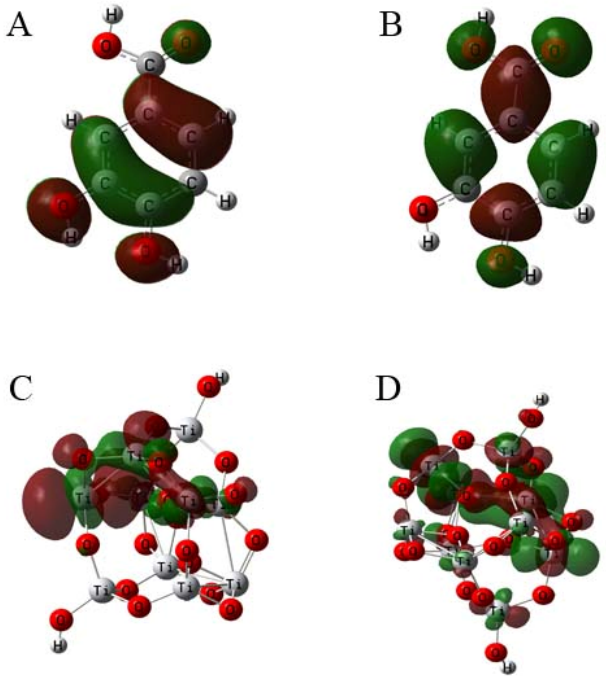


Fig.S2 HOMO (A) and LUMO (B) of optimized DB model and HOMO (C) and LUMO (D) of optimized $Ti_{10}O_{20}$ cluster

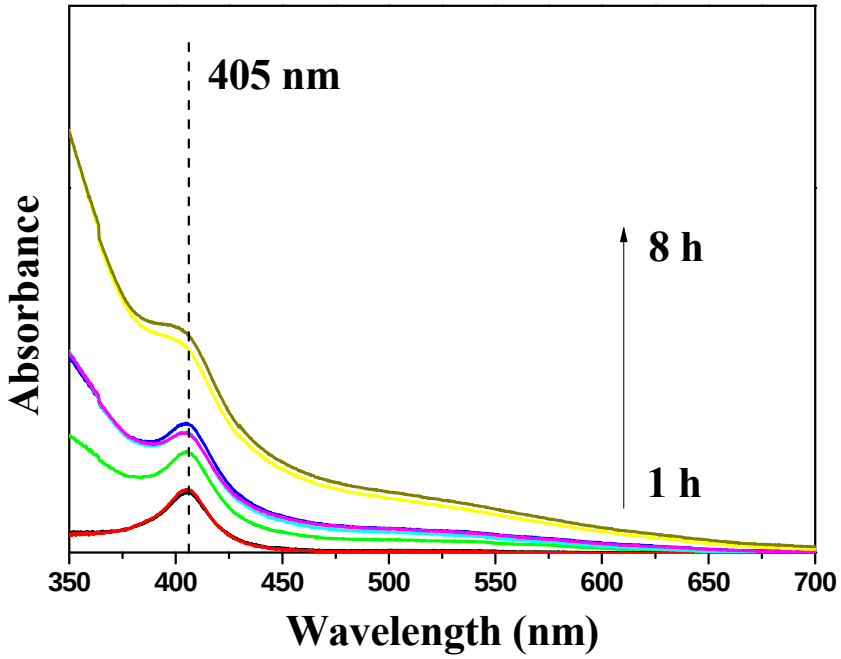


Fig.S3 Uv-vis absorption spectra of supernatant of $TiO_2$-DB-Hb at different irradiate time.

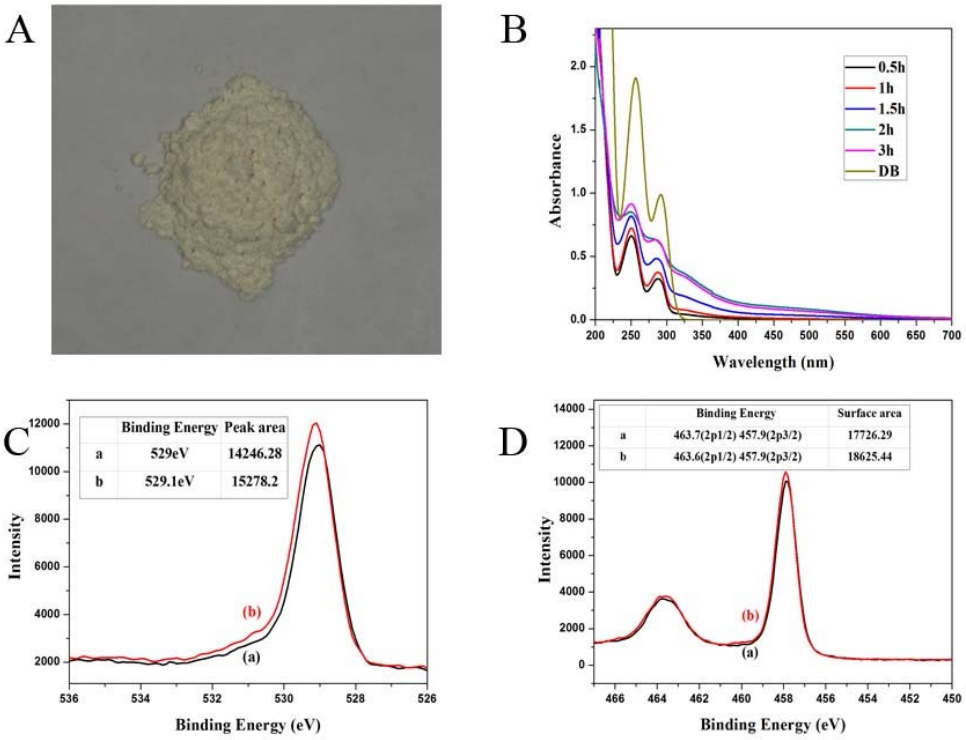


Fig.S4 (A) Photograph of $TiO_2$-DB after irradiated for 10h. (B) the Uv-vis absorption spectra of supernatants of $TiO_2$-DB suspension at different irradiate time and DB in aqueous solution. (C) O 1s high resolution XPS of (a) P25 and (b) irradiated $TiO_2$-DB. (D) Ti 2p high resolution XPS of (a) P25 and (b) irradiated $TiO_2$-DB.