# Facile and green preparation of multifeatured montmorillonite-supported $Fe_3O_4$-$Cu^{2+}$ hybrid magnetic nanomaterials for the selective adsorption of a high-abundance protein from complex biological matrices

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**Materials**

Montmorillonite (MMT) and trihydroxymethyl aminomethane (Tris, pH = 7.3, 10 mM) were purchased from Aladdin. Bovine hemoglobin (BHb) was obtained from Sigma. Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), histidine, sodium polyacrylate (PAAS), ferric chloride crystals ($FeCl_3 \cdot 6H_2O$), anhydrous sodium acetate (NaOAc), ethylene glycol (EG), diethylene glycol (DEG), hydrochloric acid (12 mol $L^{-1}$), and ethanol were provided by Xi'an Chemicals Ltd. Competitive proteins viz. bovine serum albumin (BSA), cytochrome C (Cyt C), lysozyme (Lyz), ribonuclease A (RNase A), angiogenin (ANG), myeloperoxidase (MPO), and lactoferrin (LF) were obtained from Shanghai Aladdin Ltd. The DI water (18 MΩ $cm^{-1}$) was obtained from a Water Pro water system (Axl-water Corporation, TY10AXLC1805-2, China) and used throughout the experiments. All the chemicals were of at least analytical grade and used as received.

**Characterization**

The morphological characteristics of the nanomaterials in this work were examined by field transmission electron microscope (TEM) (jem-2100, Japan) and scanning electron microscope (SEM) (JEOL Co, Japan). Fourier transform infrared (FT-IR) spectra in the region of 400-4000 $cm^{-1}$ were obtained by a Nicolet AVATAR 330 FT-IR spectrophotometer. The X-ray powder diffraction (XRD) was carried out in the $2\vartheta$ range of 3-80° by a Rigaku D/max/2500v/ pc (Japan) X-ray diffractometer with Cu Kα radiation. A vibrating sample magnetometer (VSM) (LDJ 9600−1, USA) was utilized to analyze the magnetic properties. XPS analysis was performed on an ESCALAB 250Xi X-

ray spectrometer (Thermo Fisher Scientific, Waltham, MA). All samples were analyzed using an Al Kα X-ray source (spot size of 500 μm) at a constant dwelling time for 100 ms wide scan (single scan, a step size of 1.00 eV) and 300 ms narrow scan (10 scans, a step size of 0.10 eV). The survey spectra and high-resolution single core-level spectra were measured at the pass energies of 100 and 20 eV, respectively. To neutralize the charge on the sample during the experiments, an electron-ion charge compensation system was used. The studies were carried out under ultrahigh vacuum $10^{-10}$ mbar at room temperature; in the case of using a sample charge compensation system, the partial pressure of argon in the analytical chamber was $2 \times 10^{-7}$ mbar. The experimental data were processed using ESCALAB 250 Xi spectrometer software (Avantage v5.9904, Thermo Fisher Scientific, Waltham, MA). The surface area and pore volume of the prepared nanomaterials were tested from $N_2$ adsorption/desorption by brunauer−emmett−teller (BET). The light adsorption data were obtained by using a UV-2450 spectrophotometer (Shimadzu, Japan). Electrophoretic analysis of protein samples was performed using regular SDS-PAGE (Bio-Rad, Hercules, CA) with 10% running and 5% stacking gels. Proteins were stained with Coomassie Brilliant Blue R-250.

**The definitions of the relevant parameters in formulas**

The $C_0$ and $C_e$ (mg $mL^{-1}$) are respectively the initial and equilibrium concentration of BHb. The BHb solution and the mass of the nanomaterials are expressed in $V$ (mL) and

$W$ (g), respectively. $k_1$ and $k_2$ (g $mg^{-1}$ $min^{-1}$) refer to the equilibrium rate constants. The initial rate of absorption is represented by $V_0$ (mg $g^{-1}$ $min^{-1}$). $Q_e$ is the adsorption capacity at equilibrium. $Q_t$ represents the adsorption capacity at different adsorption times, $t$ (min). The $K_F$ (mg $g^{-1}$) and $K_L$ (L $mg^{-1}$) stand for the constants of two thermodynamic models. $C_e$ (mg $L^{-1}$) represents the concentration of BHb at equilibrium. $Q_{max}$ (mg $g^{-1}$) indicates the optimum adsorption capacity of adsorbed nanomaterials, and $m$ denotes the *Freundlich* exponent.

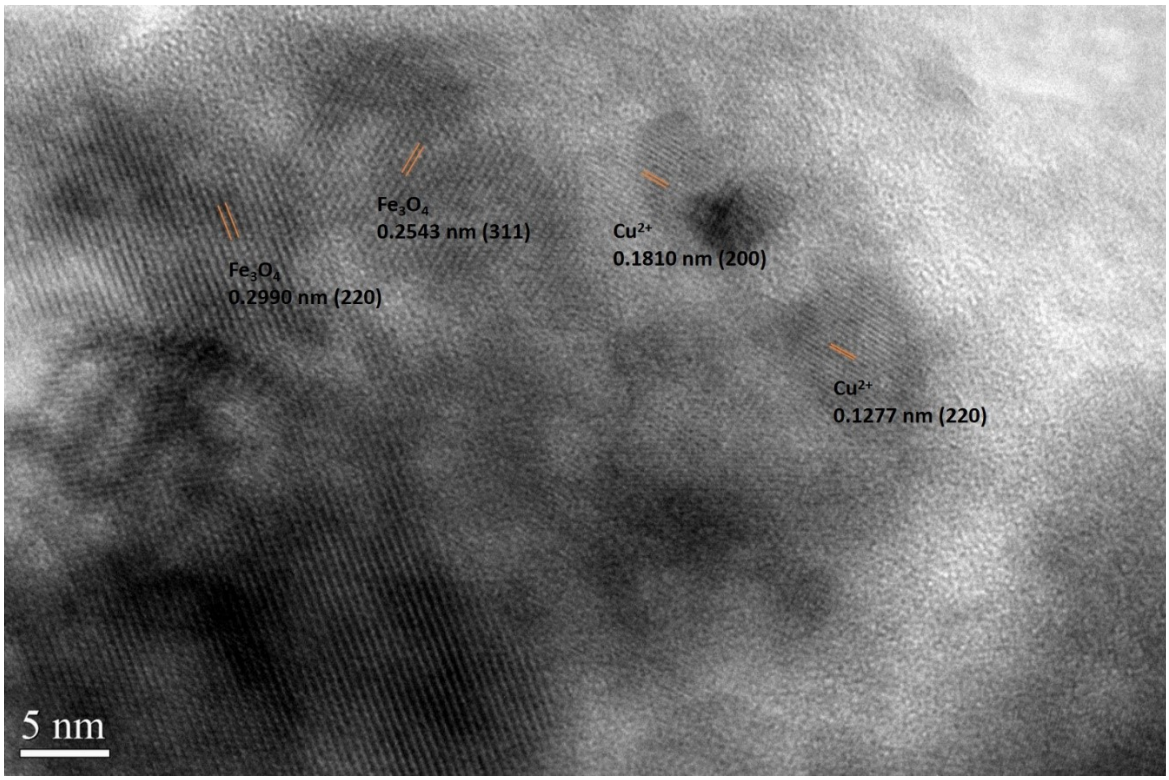


**Fig. S1.** The high-resolution TEM image for $Fe_3O_4$-$Cu^{2+}$/MMT.

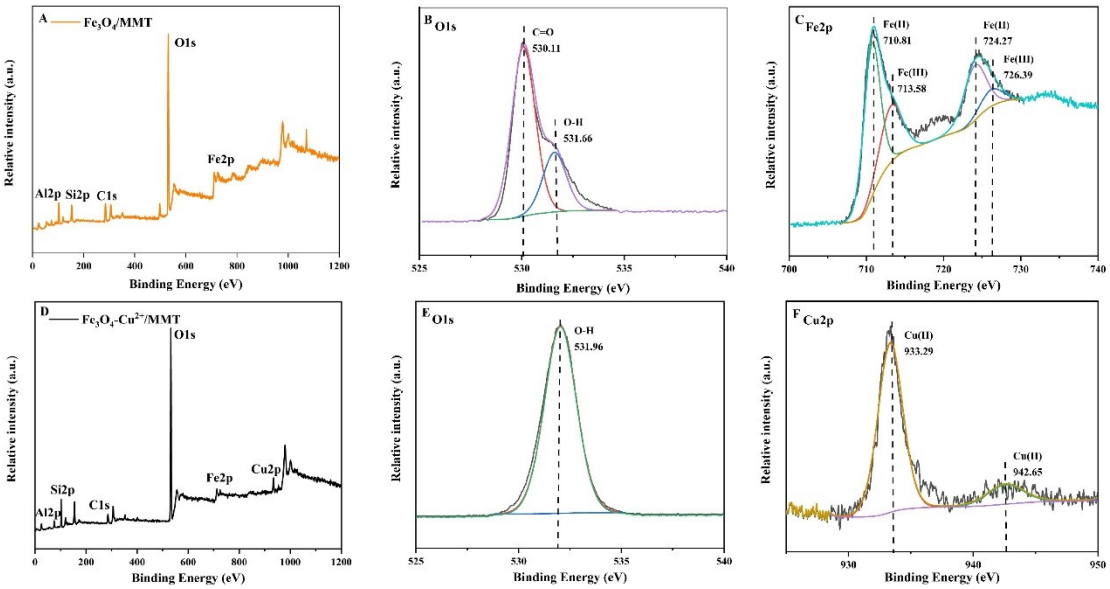


**Fig. S2.** XPS spectra of wide scan of $Fe_3O_4$/MMT (A), O 1s (B), Fe 2p (C) spectra of $Fe_3O_4$/MMT; XPS spectra wide scan of $Fe_3O_4$-$Cu^{2+}$/MMT (D), O 1s (E), Cu 2p (F) spectra of $Fe_3O_4$-$Cu^{2+}$/MMT.

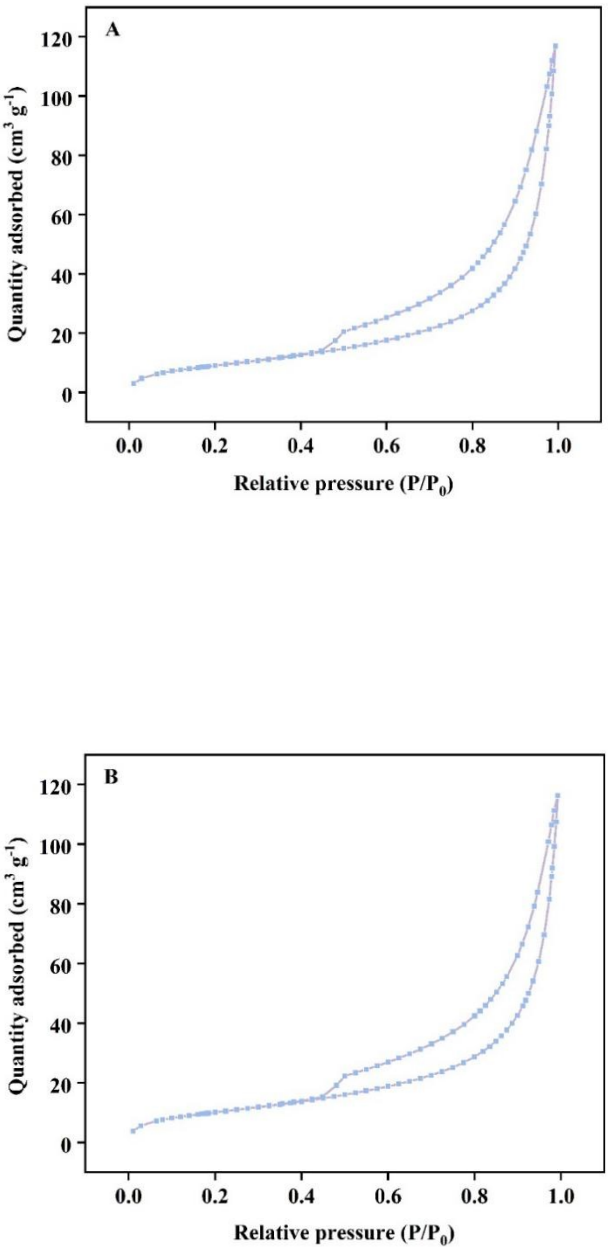


**Fig. S3.** $N_2$ adsorption/desorption isotherm of $Fe_3O_4$/MMT (A) and $Fe_3O_4$-$Cu^{2+}$/MMT (B).

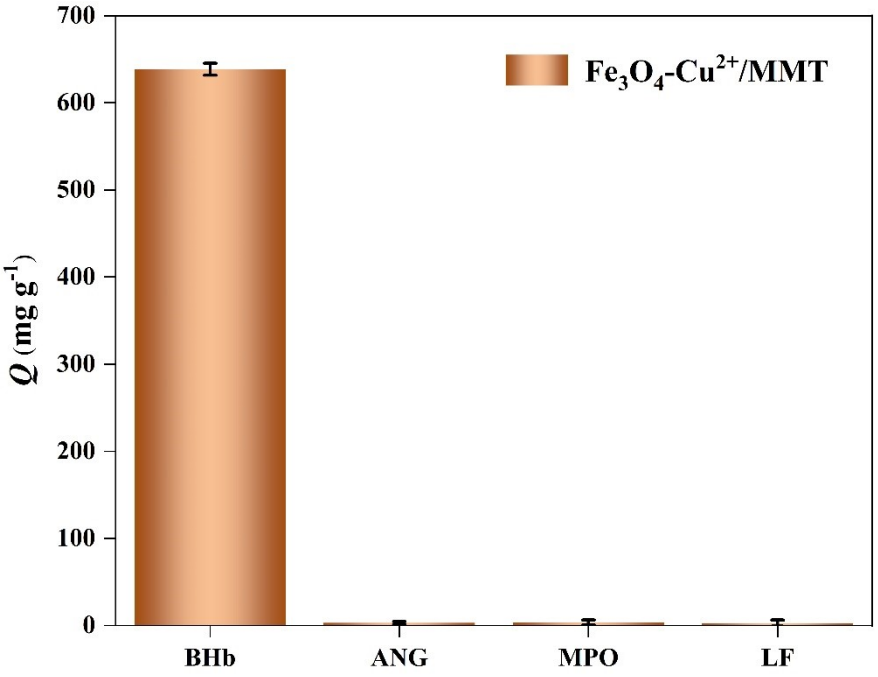


**Fig. S4.** Adsorption of BHb and low-abundance proteins by $Fe_3O_4$-$Cu^{2+}$/MMT.

**Table S1.** Equations and parameters of adsorption kinetics of $Fe_3O_4$-$Cu^{2+}$/MMT.

| Model | Equations and parameters | $Fe_3O_4$-$Cu^{2+}$/MMT |
|---|---|---|
| Pseudo-first-order | Equation | $\ln (Q_e-Q_t) = 0.07604t+6.4159$ |
| | $Q_e$ (mg $g^{-1}$) | 611.49 |
| | $K_1$ (g $mg^{-1}$ $min^{-1}$) | 0.07604 |
| | $r$ | 0.9129 |
| Pseudo-second-order | Equation | $t / Q_t = 0.00147t + 0.01152$ |
| | $Q_e$ (mg $g^{-1}$) | 680.27 |
| | $K_2$ (g $mg^{-1}$ $min^{-1}$) | 0.0137 |
| | $V_0$ (mg $g^{-1}$ $min^{-1}$) | 86.81 |
| | $r$ | 0.9904 |

**Table S2.** Equations and parameters of adsorption thermodynamic of $Fe_3O_4$-$Cu^{2+}$/MMT.

| Model | Equations and parameters | $Fe_3O_4$-$Cu^{2+}$/MMT |
|---|---|---|
| *Langmuir* thermodynamic model | Equation | $C_e / Q = 0.0014C_e + 0.0135$ |
| | $Q_{max}$ (mg $g^{-1}$) | 695.17 |
| | $K_L$ (mg $g^{-1}$) | 0.1064 |
| | *r* | 0.8591 |
| *Freundlich* thermodynamic model | Equation | $\log Q = 0.8410 \log C_e + 0.2303$ |
| | $K_F$ (mg $g^{-1}$) | 1.6995 |
| | *m* | 0.8410 |
| | *r* | 0.9901 |