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Supplementary Information

Reversing the order of changes in environmental conditions alters aggregation

behavior of hematite nanoparticles

Yuefei Ding,^{a,†} Anxu Sheng,^{a,†} Feng Liu,^a Xiaoxu Li,^a Jianying Shang,^b Juan Liu^{a,*}

^a The Key Laboratory of Water and Sediment Sciences, College of Environmental Sciences and

Engineering, Peking University, Beijing 100871, China

^bDepartment of Soil and Water Sciences, China Agricultural University, Beijing 100193, China

To be submitted to Environmental Science: Nano

*Corresponding authors.

E-mail address: juan.liu@pku.edu.cn (Juan Liu)

[†]Both authors contributed equally to this work

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Section S1. Synthesis and characterization procedures of hematite nanoparticles

Hematite nanoparticles were synthesized according to the method reported by Schwertmann and Cornell.¹ In brief, 4.04 g Fe(NO₃)₃·9H₂O was dissolved in 500 mL of 0.002 M HCl solution under vigorous stirring in a screw-cap bottle. After that, suspension was heated in an oven at 98 °C for 7 days. The synthetic hematite nanoparticles were dialyzed in standard grade cellulose dialysis membranes (1000 MWCO, Spectra/Por, USA) against Milli-Q water until the conductivity kept constant at ~ 5 μ S/cm.

The mass concentration of hematite nanoparticle in suspension was measured by acid digestion method. After sonication for 10 minutes, 0.1 mL nanoparticle suspension was dissolved in 9.9 mL of 5 M HCl solution and continuously shaken overnight. Then 0.02 mL of digested solution was diluted with 2% HNO₃ to 5 mL.² The concentration of Fe was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Prodigy ICP, Teledyne Leeman Labs, USA).

Primary particle size and morphology of hematite nanoparticles were measured on a fieldemission transmission electron microscope (TEM, JEM-2100F, JEOL, Japan) operated at 200 kV. TEM samples were prepared by dropping diluted hematite suspension onto a 400 mesh copper grid coated with ultrathin carbon layer, and then drying it in air at room temperature. More than 100 hematite nanoparticles on TEM images were randomly selected and analyzed using ImageJ software to obtain the primary particle size distribution.³ XRD sample was prepared by depositing concentrated hematite stock suspension onto a glass slide and dried in air at room temperature. The crystalline phase of hematite nanoparticles was characterized by powder X-ray diffraction (XRD) using a Rigaku D/MAX-2000 diffractometer with monochromatic CuK α radiation ($\lambda = 0.15406$ nm) at a scan rate of 0.02 $2\theta \cdot s^{-1}$.

Section S2. Determination of aggregation kinetics in different exposure orders

The particle attachment efficiency α , ranging from 0 to 1 and quantifying the aggregation kinetics of hematite NPs, was determined by normalizing the *k* obtained under each stage (linear fitting 60 measurements data in the Stage 1 and 2) of different exposure orders (BSA \rightarrow NaCl, NaCl \rightarrow BSA) by the k_{fast} obtained under favorable (fast) aggregation conditions^{2, 4}:

$$\alpha = \frac{k}{k_{\text{fast}}} = \frac{\frac{1}{N_0} \left(\frac{dD_h(t)}{dt}\right)_{t \to 0}}{\frac{1}{(N_0)_{\text{fast}}} \left(\frac{dD_h(t)}{dt}\right)_{t \to 0,\text{fast}}}$$

To calculate the attachment efficiency (α) values in the experiments with BSA, k_{fast} was obtained from the hematite suspensions without BSA at high ionic strength greater than the critical coagulation concentration (CCC).²

Section S3. Calculation of the EDL thickness of hematite nanoparticle

The thickness of the electrical diffuse layer (EDL) of nanoparticle was calculated according to the equation describing the correlation between ionic strength and the Debye-Hüchel length (k^{-1} , nm) in the following equation⁵:

$$\kappa^{-1} = \sqrt{\frac{\varepsilon\varepsilon_0 k_B T}{2N_A I e^2}}$$

where ε is the dielectric constant of water, ε_0 is the permittivity of vacuum, k_B is the Boltzmann constant, T is temperature, N_A is Avogadro constant, e is the elementary charge, and I is the ionic strength.



Fig. S1 Schematic diagram of the method used to analyze the aggregation behavior of hematite nanoparticles in three exposure orders of protein and salt ions. These exposure orders include:
1) simultaneous addition of NaCl and BSA (BSA+NaCl); 2) addition of BSA first and then NaCl (BSA→NaCl); 3) addition of NaCl first and then BSA (NaCl→BSA).



Fig. S2 Aggregation profiles of hematite nanoparticles as a function of BSA (16, 24 and 32 mg/L) and NaCl (100 mM) concentration. The samples were shaken between stage I and II without adding extra NaCl or BSA in stage II.



Fig. S3 Deconvolution of amide I band in the FTIR spectrum of adsorbed BSA on hematite particles to obtain the relative proportions of protein secondary structure features, including α -helix (1650-1655 cm⁻¹), β -turn (1660-1690 cm⁻¹), β -sheet (1620-1635 cm⁻¹), and random coil (1644-1648 cm⁻¹).



Fig. S4 XRD pattern of synthetic hematite nanoparticles (above) and reference hematite pattern (below, JCPDS No.433-0664).



Fig. S5 Representative transmission electron microscopy (TEM) image of synthetic hematite nanoparticles (scale bar is 100 nm) (a). Statistically analyzed size distribution diagram indicates the primary particle size of 41 ± 8 nm (b).



Fig. S6 Aggregation profiles (a) and attachment efficiencies (b) of hematite nanoparticles as a function of electrolyte concentration at pH 5.7.



Fig. S7 Zeta potentials of hematite nanoparticles as a function of NaCl (a) or BSA (b) concentration at pH 5.7.



Fig. S8 Aggregation profiles of hematite nanoparticles as a function of BSA concentration at 10 mM NaCl in different exposure orders of protein and electrolyte, including BSA+NaCl (a), BSA→NaCl (b), and NaCl→BSA (c).



Fig. S9 Zeta potentials of hematite nanoparticles as a function of BSA concentration at 10 mM NaCl (a) and 100 mM NaCl (b) in different exposure orders of protein and electrolyte, including BSA+NaCl, BSA \rightarrow NaCl, and NaCl \rightarrow BSA. The black box represented the zeta potentials of the BSA-hematite conjugates without electrolyte. Zeta potential measurements were conducted at pH 5.7 and 25 °C.



Fig. S10 Aggregation profiles of hematite nanoparticles as a function of BSA concentration at 100 mM NaCl in different exposure orders of protein and electrolyte, including BSA+NaCl (a), BSA→NaCl (b), and NaCl→BSA (c).



Fig. S11 The amounts of adsorbed BSA on hematite nanoparticles (64 mg/L) in the experiments with the exposure orders of BSA+NaCl (black), BSA \rightarrow NaCl (red), and NaCl \rightarrow BSA (blue) at low (left) and high (right) ionic strength, respectively. The initial BSA concentration was 48 mg/L.



Fig. S12 Representative time-dependent FTIR spectrum of adsorbed BSA macromolecules on the surface of hematite nanoparticles.



Fig. S13 Time-dependent relative proportions of secondary structure features, including α -helix (blue), random coil (red), β -sheet (black), and β -turn (green), in adsorbed BSA macromolecules at 0 mM NaCl (a), 10 mM NaCl (b), and 100 mM NaCl (c), respectively.



Fig. S14 The equilibrium Cu(II) uptake capacity on hematite-protein conjugates ([BSA]= 24 mg/L and 80 mg/L) at 10 mM NaCl in different protein/electrolyte exposure orders at pH 5.7 after 2 h adsorption. And the final hydrodynamic diameters of hematite aggregates in the equivalent experimental conditions after 20 minutes reaction time were also showed.

References

1. U. Schwertmann, Iron oxides in the laboratory: preparation and characterization, 2nd completely revised and enlarged edition, *Mineral. Mag.*, 2000, **61**, 740-741.

2. A. Sheng, F. Liu, N. Xie and J. Liu, Impact of proteins on aggregation kinetics and adsorption ability of hematite nanoparticles in aqueous dispersions, *Environ. Sci. Technol.*, 2016, **50**, 2228-2235.

3. J. Liu, D. M. Aruguete, J. R. Jinschek, J. Donald Rimstidt and M. F. Hochella, The nonoxidative dissolution of galena nanocrystals: Insights into mineral dissolution rates as a function of grain size, shape, and aggregation state, *Geochim. Cosmochim. Acta*, 2008, **72**, 5984-5996.

4. A. Sheng, F. Liu, L. Shi and J. Liu, Aggregation kinetics of hematite particles in the presence of outer membrane cytochrome OmcA of Shewanella oneidenesis MR-1, *Environ. Sci. Technol.*, 2016, **50**, 11016-11024.

5. W. Zhang, in *Nanomaterial. Advances in Experimental Medicine and Biology*, Springer, 2014, vol. 811, pp. 19-43.