Materials and Methods

Materials

All chemical reagents were obtained from commercial sources and used without any further purification. Gram-positive bacteria (*Staphylococcus aureus*; ATCC 6538) and the mouse monocyte macrophage RAW 264.7 cell line (ATCC®TIB-71) were obtained from were purchased from American Type Culture Collection. Tryptic Soy Broth (BD 211825) powder and LIVE/DEAD viability kit were purchased from Fisher Scientific. Both normal human dermal fibroblasts (NHDF-Ad) and human umbilical vein endothelial cells (CC-2935) were purchased from Lonza.

Measurement methods

Fourier transform infrared (FTIR) spectroscopic data were obtained on a Bruker Tensor 27 Instrument using a KBr matrix in the 4000-400 cm^{-1} region. The thermal analysis was conducted on a bulk sample using a TA instruments 2950 high-resolution thermogravimetric analyzer (New Castle, DE, USA) in air from room temperature to 800 °C with a heating rate of 10 °C/min. Samples for Transmission electron microscope (TEM) were placed as droplets onto a carbon-coated nickel TEM grid (400-mesh). Specimens were dried in air and analyzed at 200 kV using a FEI Tecnai F20 transmission electron microscope (TEM) equipped with a field emission gun. The energy dispersive X-ray spectroscopy (EDX) results were obtained with the integrated scanning TEM (STEM) unit and attached EDAX spectrometer. The spatial resolution is <1 nm through the acquisition of high resolution (~0.2 nm) high-angle angular dark field (HAADF) images, which is sensitive to atomic number (Z) contrast. The metal ratio analysis was performed by AAS, in which the NPs sample of 10 mg was first disgested by 75 % HNO_3. Then, the substrate was decomposed at 620 °C for 5 hours, and the metal oxides obtained were dissolved in *aqua regia* and diluted to the linear dynamic range of AAS before analysis.

Synthesis of PVP-coated CaPB NPs

A solution of 10.0 mM $K_3[Fe(CN)_6]$ (20 mL, pH ≈ 1) was added dropwise to a solution of 10.0 mM $CaCl_2$ (20 mL) containing 500 mg of PVP (average MW = 40,000) at room temperature to give a clear pale-yellow solution. After stirring for 20 min, the solution was transferred into a dialysis bag made of regenerated cellulose tubular membrane (MWCO is 12,000-13,000) and dialyzed against distilled water for 2 hours. The distilled water used in the dialysis was changed every 30 min. The solid product was collected by overnight lyophilization.

Preparation of the bulk $KCa[Fe(CN)_6]$ sample

Bulk $K[CaFe(CN)_6]$ samples were prepared using solutions of $K_3[Fe(CN)_6]$ and $CaCl_2$ in the absence of a coating agent. Specifically, a 10-mL aqueous solution of $CaCl_2$ (5.0 mM) was added to a 10-mL $K_3[Fe(CN)_6]$ aqueous solution (5.0 mM, pH ≈ 1) under vigorous stirring at room temperature. This reaction resulted in a pale yellow turbid solution. After stirring for 20 min at room temperature, the product was purified by dialysis against distilled water for 2 hours and the solid product was collected by lyophilization.

Crystal growth of $(H_3O)Ca(H_2O)_2[Co(CN)_6]$

Preparation of $(H_3O)Ca(H_2O)_2[Co(CN)_6]$ was carried out by the reaction of $H_3[Co(CN)_6]$ (2.18 g, 10 mmol) with $CaCl_2$ (1.11 g, 10 mmol) in aqueous solution. Pale-yellow crystals were collected through slow evaporation of water at room temperature after one week.

Single-crystal structure determination of $(H_3O)Ca(H_2O)_2[Co(CN)_6]$

The X-ray single-crystal diffraction data were collected on a Rigaku Saturn 724$^+$ diffractometer with Mo–Kα radiation (λ = 0.71073 Å) at 298 K. Data processing including empirical absorption corrections was performed using the *CrystalClear* software package (Rigaku, 2005). The structure was solved by direct methods and refined by the full-matrix method based on F^2 using the *SHELXLTL* software package. Non-H atoms were refined anisotropically using all reflections with $I > 2\sigma$ (I). All H atoms were generated geometrically and refined using a "riding" model with $U_{iso} = 1.2U_{eq}$ (C and N). Crystallographic data and structure refinement are listed in Table S1. Angles and distances between some selected atoms were listed in **Tables S2** and **S3**.

Solution ion-exchange studies

A 5-mL nanoparticle suspension (5.0 mM) was sealed in a dialysis bag (MWCO=12,000-13,000), which was then brought in contact with a $FeCl_2$ solution (60 mL) with the initial concentration at ~10 ppm level. The iron concentrations of the solution outside the dialysis bag were periodically analyzed by AAS to give the kinetic data given in **Table S4**. The raw data can be fitted to two separate rate laws, i.e. the *pseudo* first order and the second order reactions with the first order rate constant $k_1 = 2.1\times10^{-4}$ s^{-1} and the second order rate constant of $k_2 = 9.2\times10^{-2}$ M^{-1} s^{-1} (**Figures S4** and **S5**).

Antibacterial activity evaluation

One *S. aureus* colony was first cultured in fresh tryptic soy broth (TSB) medium (5 mL) at 37 °C and 180 rpm overnight. Then, 50 μL of the cultured suspension was added to 5 mL of fresh TSB, which was incubated at 37 °C for 4 hours in a shaker at 180 rpm. The CFU (colony-forming unit) of the starting bacterial suspension was approximately 10^9 per mL. The specific test method is as follows: a 100-μL bacterial suspension was added to a volume of 880 μL TSB medium, and then treated with a 20-μL solution containing a given amount of CaPB NPs. After 24-hour incubation, a portion of this bacterial solution (10 μL) was taken out and diluted 10^6 times. Next, 50 μL of the diluted suspension was spread in an agar plate. After incubation for 24 hours at 37 °C, the CFU values were obtained by counting the colonies in the agar plate. All measurements were performed in triplicate.

Metal quantification for monitoring the cellular uptake of CaPB NPs

The metal uptake was performed using AAS to determine the calcium and iron concentrations between experimental and control groups. Briefly, based on the antibacterial activity testing described in the above experimental method, a dose of 100-μM CaPB NPs was selected to treat bacterial cells for the NP uptake studies. After incubated for 6 hours, a 500-μL aliquot of NP-treated cell suspension was withdrawn, and CFU was determined by agar plate counting. The remaining bacterial cells were collected by centrifuging (3700 rpm) at 25 °C for 7 min.

The supernatant was discarded and the pellet was washed with deionized water three times. Then, the bacterial pellet was digested using 75% HNO_3 to destroy the organics, and the metal ions in this solution were then converted to oxides by calcination of the sample at 620 °C for 5 hours. The metal oxides obtained were dissolved in aqua regia and the calcium and iron concentrations were analysed using AAS.

Iron rescue studies

Each 100-μL bacterial suspension was added to 880 μL TSB medium. Such suspensions were then each treated with a 20-μL solution containing a varying amount of CaPB NPs. After incubated for 2 hours, a volume of 20-μL solutions with an amount of $FeCl_2$ corresponding to 0-, 1-, 2-, and 5-equivalent amounts of CaPB NPs that were used to treat the cells in the previous step was added into the individual bacterial suspensions, respectively. After incubated overnight, the optical density (OD_{600}) for each suspension was measured. All measurements were performed in triplicate.

MTT assays

Cytotoxicity of CaPB NPs toward mammalian cells was determined using an MTT viability assay. Mammalian cells (RAW cells, normal human dermal fibroblasts or human umbilical vein endothelial cells) were seeded in a 96-well plate at a density of 4×10^5 cells per well with the DMEM high-glucose medium and incubated for 24 hours at 37 °C in an atmosphere of 5 % CO_2 and 95 % air to allow cells to attach to the surface. Cells in each well were then treated with 100 μL of fresh medium containing varying concentrations of the CaPB NPs and then incubated for 24 hours. Control wells contained the same medium without CaPB NPs. After that, the cells were incubated with 10 μL of MTT reagent for 2 hours at 37 °C. Then, 100 μL of detergent reagent was added to all wells and the plate left with cover in the dark for 2 hours at 37 °C. The absorbance was measured at 570 nm using a microplate reader. The assay was run in triplicate, and the results were presented as percentage of viable cells.

The LIVE/DEAD assay

The solution of fluorescent dyes was prepared by mixing 3 μL of SYTO 9 stain and 3 μL of propidium iodide stain to 1 mL of filter-sterilized water, and used for cell staining in the same day to ensure freshness. After bacterial cells with treated with 200-μM CaPB NPs and incubated at 37 °C and 180 rpm for 24 hours, the upper bacterial suspension and the lower substrate were separated. The lower substrate was washed with PBS three times. The upper suspension was centrifuged and the substrate was washed with PBS three times. Then, 200 μL of the staining solution was added into 1 mL of the upper and lower PBS solutions, respectively. After covering the staining eppendorf tubes and incubating the samples for 20-30 min at room temperature in the absence of light, both the sample and control groups were rinsing with PBS, the fluorescence images were collected using a Leica TCS SP5 II confocal microscope.

Statistics

Values are reported as means ± standard error. Metal uptakes, bacterial killing, and iron rescue data were compared to that of the control group using single or two-way ANOVA.

Differences between groups were considered significant if $P \leq 0.05$.

Table S1 Crystal data and structure refinements for $(H_3O)Ca(H_2O)_2Co(CN)_6$ at 298 K.

T/K	298
formula	$C_6H_4CaCoN_6O_2.H_3O$
Mw	310.19
crystal system	orthorhombic
space group	*Pnma* (No. 62)
a/Å	12.940(3)
b/Å	13.697(3)
c/Å	7.3605(15)
α/°	90
β/°	90
γ/°	90
V/Å^3	1304.5(5)
Z	4
ρ_{calcd}/g cm^{-3}	1.579
μ/mm^{-1}	1.71
Refs collected / unique	8284 / 1560
R_1[a], wR_2[b] ($I > 2\sigma(I)$)	0.082, 0.23
GOF	1.17
$\Delta\rho$[c]/e Å^{-3}	0.144, −0.163

[a]$R_1 = \Sigma||F_o|-|F_c||/\Sigma|F_o|$. [b]$wR_2 = [\Sigma w(F_o^2-F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$. [c]Maximum and minimum residual electron density.

Table S2 Hydrogen bonds of $(H_3O)Ca(H_2O)_2Co(CN)_6$ at 298 K.

D−H···A	D−H	H···A	D···A	∠D−H···A
O3—H4A...O2#1	0.90	2.03	2.883 (9)	156.6
O3—H4C...N3#2	0.90	2.37	3.272 (10)	177.1
O3—H4B...N2#3	0.90	2.41	3.305 (10)	172.0
O1—H1B...O3#4	0.85	2.11	2.845 (9)	144.7
O2—H2B...O3#5	0.85	2.09	2.883 (9)	155.3

Symmetry codes: #1 -x+1, -y+1, -z+1; #2 x+1/2, y, -z+3/2; #3 x, y, z+1; #4 -x+1/2, -y+1, z-1/2; #5 -x+1, y-1/2, -z+1.

Table S3 Selected bond lengths [Å] and angles [°] for $(H_3O)Ca(H_2O)_2Co(CN)_6$ at 298 K.

C(1)-N(1)	1.139(9)	C(1)-Co(1)	1.905(6)
C(2)-N(2)	1.152(8)	C(2)-Co(1)	1.893(7)
C(3)-N(3)	1.151(8)	C(3)-Co(1)	1.899(7)
Ca(1)-N(2)#1	2.511(6)	Ca(1)-N(1)	2.518(6)
Ca(1)-N(3)#2	2.526(6)	Ca(1)-O(1)	2.562(8)
Ca(1)-O(2)	2.718(9)		
N(1)-C(1)-Co(1)	177.5(7)	N(2)-C(2)-Co(1)	178.9(6)
N(3)-C(3)-Co(1)	178.6(6)	C(1)-N(1)-Ca(1)	176.5(6)
C(2)-N(2)-Ca(1)#3	155.0(6)	C(3)-N(3)-Ca(1)#4	157.4(5)
N(1)-Ca(1)-O(2)	138.73(16)	N(1)-Ca(1)-O(1)	77.1(2)

O(1)-Ca(1)-O(2)	122.5(3)	N(2)#1-Ca(1)-O(2)	69.8(2)
N(3)#2-Ca(1)-O(1)	70.70(19)	N(2)#1-Ca(1)-O(1)	141.47(15)

Symmetry codes: #1 -x+1/2,y-1/2,z-1/2; #2 x+1/2,y,-z+1/2; #3 -x+1/2,-y+1,z+1/2; #4 x-1/2,y,-z+1/2.

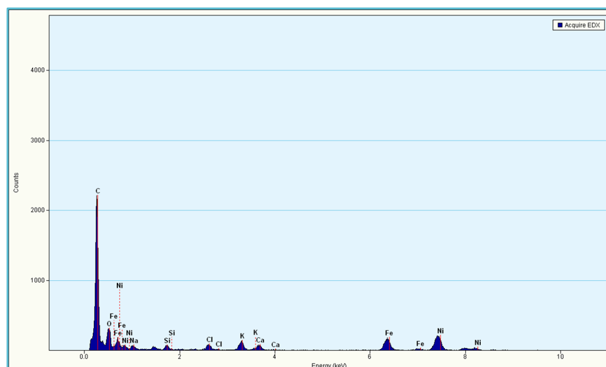

Figure S1. The EDX spectrum of a typical PVP-coated CaPB NP (Note that the nickel peak in the spectrum is from the niclel grid used for the TEM imaging studies)

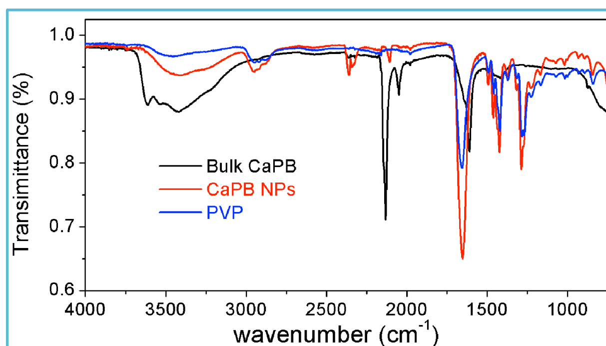

Figure S2. FT-IR spectra of PVP-coated CaPB NPs measured in comparison with those of the bulk CaPB sample and PVP

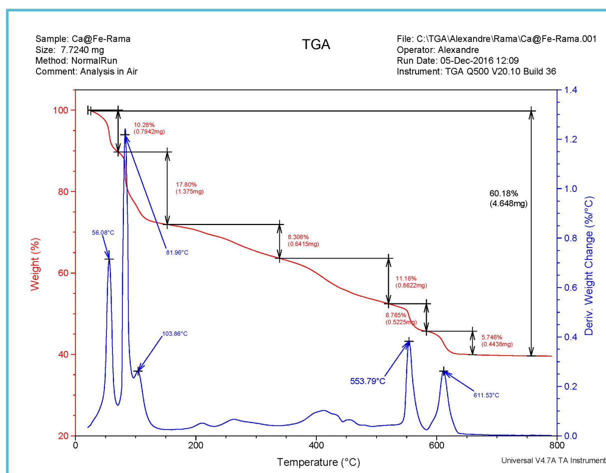

Figure S3. The TGA curve of the bulk CaPB sample

Table S4 The removal of Fe^{2+} ions and the release of Ca^{2+} ions *vs*. time in the ion-exchange reaction.

Time (min)	Fe^{2+} (μM)	Ca^{2+} (μM)
0	172.6318381	8.586494
5	143.2536485	15.97651
10	129.264034	17.03223
20	113.8754589	22.66271
30	104.082729	25.47796
50	92.89103769	29.34892
80	80.30038499	33.21988
120	70.50765512	39.20227
180	57.91700242	45.53657
240	48.12427254	51.87087
360	36.93258125	60.66851
600	31.33673561	70.16996
720	29.9377742	73.68901
1080	28.53881279	80.72712
1440	27.13985137	86.0057
1680	27.13985137	87.76523

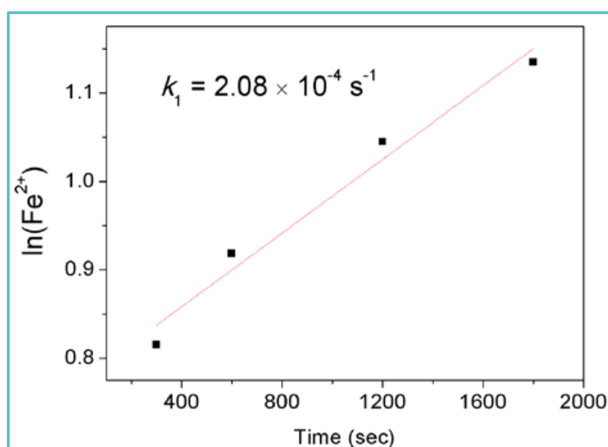

Figure S4. Curve fitting results to the *pseudo* first-order reaction for the ion-exchange reaction

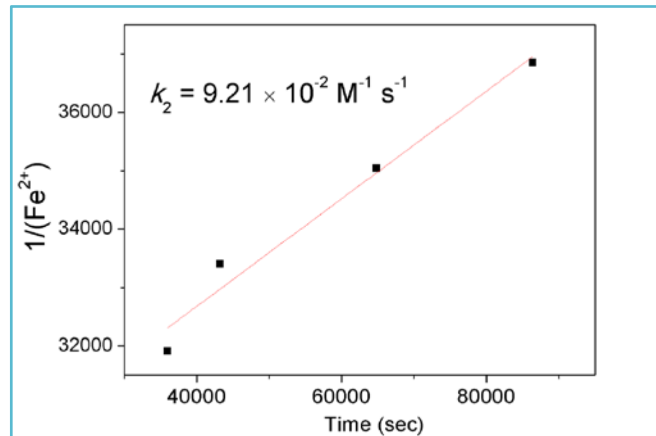

Figure S5. Curve fitting results to the pseudo second-order reaction for the ion-exchange reaction

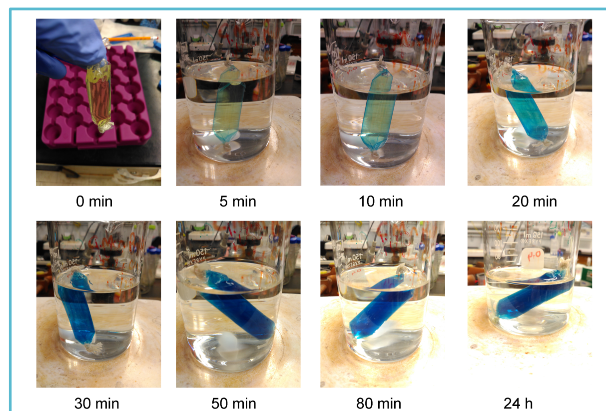

Figure S6. Visualization of colour change in the process of ion exchange

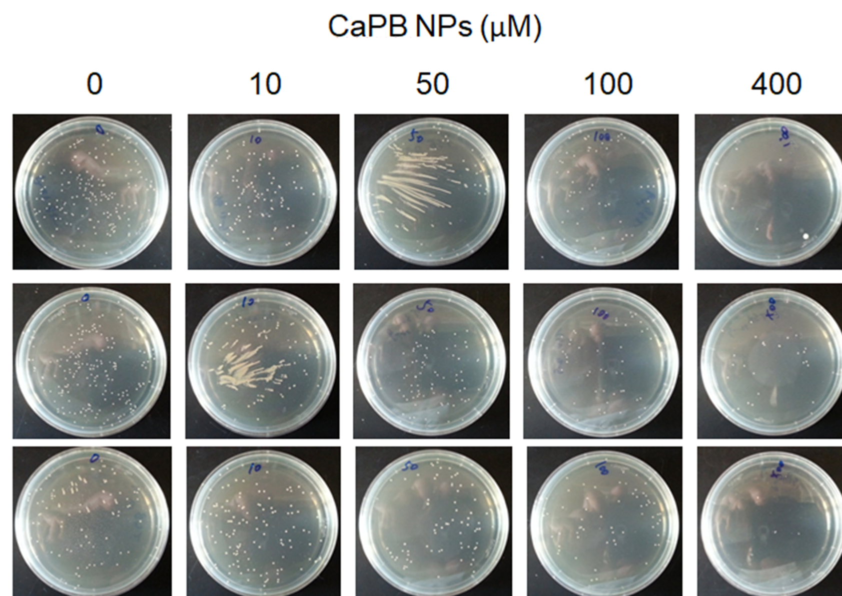

Figure S7. Representative CFU counting results in *S. aureus* cells treated with different concentrations of CaPB NPs

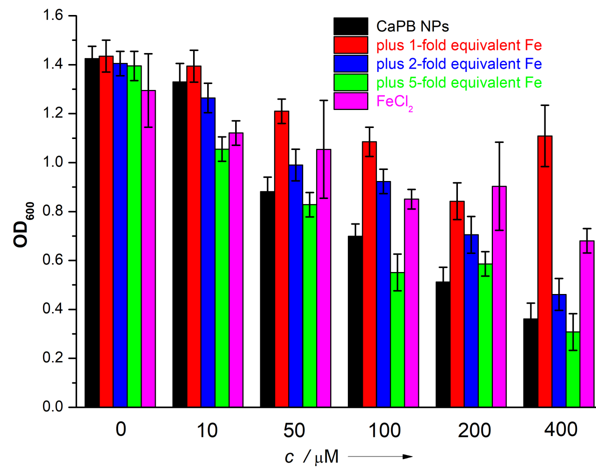

Figure S8. OD_{600} of *S. aureus* cells incubated with CaPB NPs alone (black), iron alone (magenta) and with CaPB NPs followed by addition of iron to the cell culture after 2-hour incubation (plus 1-fold equivalent Fe, red; plus 2-fold equivalent Fe, blue; plus 5-fold equivalent Fe, green)

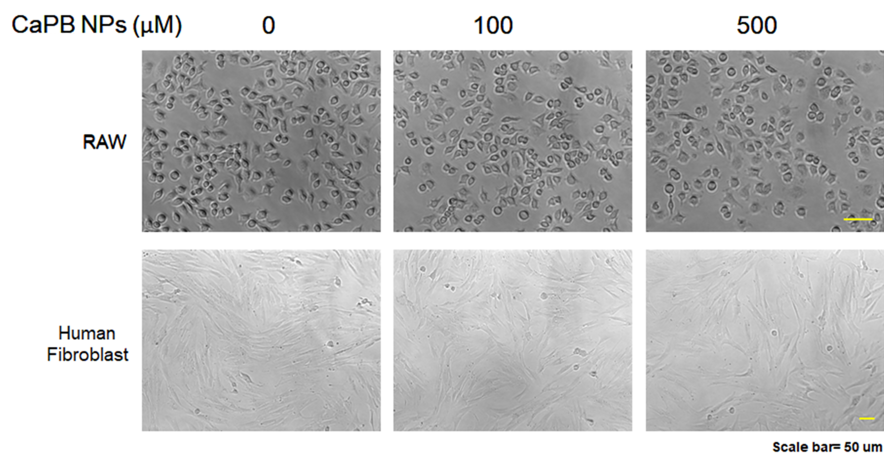

Figure S9. Representative cell morphology images after incubated with various amounts of CaPB NPs