Supporting Information

Using Magnetic Ions to Probe and Induce Magnetism of Pyrophosphates, Bacteria, and Mammalian Cells

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Abstract

Magnetic isolation using magnetic nanoparticles (MNPs) as trapping probes have been widely used in sample pretreatment to shorten analysis time. Nevertheless, to generate MNPs is time-consuming. Furthermore, the generated MNPs have to be further functionalized to gain the capability of recognizing their target species. Thus, an alternative approach that can impose magnetism to non-magnetic species by simply using magnetic ions as the probes is developed in this study. That is, we employ magnetic ions (Fe^{3+}, Co^{2+}, and Ni^{2+}) that can interact with non-magnetic species containing oxygen-containing functional groups as the probes. Pyrophosphate (PPi), bacteria, and mammalian cells were selected as the model samples. Our results show that the as-prepared magnetic ion-PPi conjugates gain sufficient magnetism and can be readily aggregated by applying an external magnetic field. Moreover, the magnetic trapping is reversible. The PPi containing conjugates can lose their magnetic property simply using ethylenediaminetetraacetic acid or aluminum ions as competing agents to remove or to replace, respectively, the conjugated magnetic ions. In addition, bacteria and mammalian cells that possess abundant oxygen-containing functional groups on their cell surfaces can be selectively probed by magnetic ions and gain sufficient magnetism for magnetic isolation from complex serum samples.

Additional Experimental Details

Reagents and Materials

Sodium pyrophosphate (PPi) tetrabasic decahydrate, cobalt chloride hexahydrate, ethylenediaminetetraacetic acid disodium salt dehydrate, and iron chloride hexahydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nickelchlorid-6-hydrate and aluminum chloride hexahydrate were purchased from Riedel-de Haën (Buchs, St. Gallen, Switzerland). Tris(hydroxymethyl)aminomethane and tris hydrochloride were obtained from J. T. Baker (Phillipsburg, NJ, USA). Tryptic soy broth (TSB) was purchased from Becton Dickinson (Franklin Lakes, NJ, USA). Yeast extract (YE) was purchased from Group Research Tech. Agarose was purchased from Amresco (Solon, OH, USA). Fetal bovine serum was purchased from Biological Industries (Cromwell, CT, USA). *Escherichia coli* O157:H7 (BCRC 13085) and breast cancer cells (T47D, BCRC 60250) were purchased from Bioresource Collection and Research Center (Hsinchu, Taiwan). *Staphylococcus aureus* was collected from the patients in a Tzu-Chi General Hospital (Hualien, Taiwan) and provided by Prof. P.-J Tsia. A neodymium magnet (~4000 Gauss) was obtained from a local shop.

Instrumentation

Hysteresis curves were obtained using a superconducting quantum interference device magnetometer (SQUID) (MPMS XL-7) from Quantum Design (San Diego, CA, USA). Scanning electron microscopy (SEM) images were obtained from a JEOL JSM-7401F scanning electron microscope.

Preparation of Bacterial Samples

E. coli O157:H7 and *S. aureus* were cultured in TSBY broth (10 mL), which was prepared by mixing TSB (3 g mL^{-1}) and YE (0.5 g mL^{-1}) in deionized water (400 mL).

The bacteria cells were harvested after incubated at 37 ℃ for 12 h. The harvested bacterial cells were isolated after centrifugation at 6000 rpm (rotor radius: 8.0 cm) for 5 min using a Thermo D-37520 centrifuge (Waltham, MA, USA). The collected bacterial cells were rinsed with deionized water (1 mL×2) followed by centrifugation at 6000 rpm 5 min. The resultant bacterial cells were re-suspended in deionized water (1 mL), and heated to boil for 1 h. The resultant bacterial cells were lyophilized and stored for future use. Prior to mixing with magnetic ions, bacterial samples were prepared in Tris buffer (20 mM, pH 7.4) with a given concentration.

Preparation of SQUID Samples

When conducting SQUID analysis, the metal ion-PPi complexes were prepared by vortex-mixing aqueous metal chloride including $FeCl_3$, $CoCl_2$, and $NiCl_2$ (0.1 M, 0.1 mL) individually with PPi (0.04 M, 0.1 mL) in deionized water for 30 min. The resulting precipitates composed of metal ion-PPi complexes were rinsed by deionized water (0.2 mL×3) followed by centrifugation at 10,000 rpm for 10 min. The resultant metal ion-PPi complexes were lyophilized to remove water prior to SQUID analysis

Additional Results

Table S1. The temperatures of the samples (0.2 mL) containing *S. aureus* (2 mg mL^{-1} (OD_{600}=1 (~0.64 mg mL^{-1})) and magnetic ions (0.1 M) obtained after heated in a microwave oven (power: 180 W, 90 s $cycle^{-1}$ × 3). The starting temperature was ~25 ^{o}C. Three replicates were conducted.

Replicates	Fe(III)	Co(II)	Ni(II)
1	30.3 ^{o}C	29.7^{o}C	31.4 ^{o}C
2	29.7 ^{o}C	30.1^{o}C	31.8 ^{o}C
3	30.4 ^{o}C	31.6 ^{o}C	30.0 ^{o}C

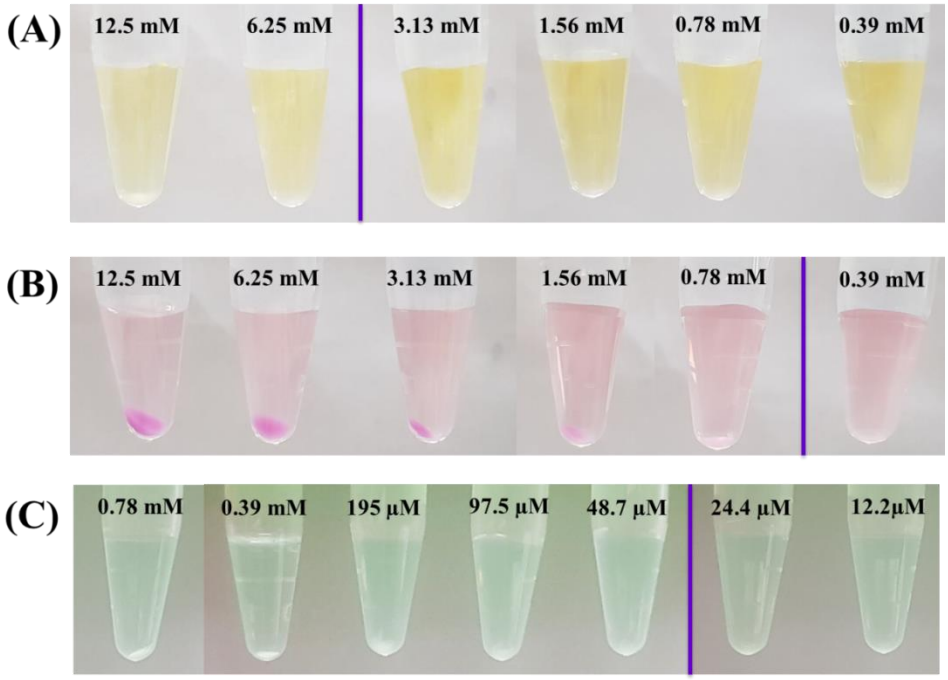

Figure S1. Estimation of solubility product constant (k_{sp}). (A) Aqueous $FeCl_3$ (0.2 M, 0.2 mL) mixed with different concentrations of PPi (0.2 mL) for 30 min followed by centrifugation at 10,000 rpm for 10 min. (B) Aqueous $CoCl_2$ (0.2 M, 0.2 mL) mixed with different concentrations of PPi (0.2 mL) for 30 min followed by centrifugation at 10,000 rpm for 10 min. (C) Aqueous $NiCl_2$ (0.2 M, 0.2 mL) mixed with different concentrations of PPi (0.2 mL) for 30 min followed by centrifugation at 10,000 rpm for 10 min. The concentrations labeled on the vails denoted the final concentrations of PPi in the mixtures. The purple line indicated where precipitates started to disappear.

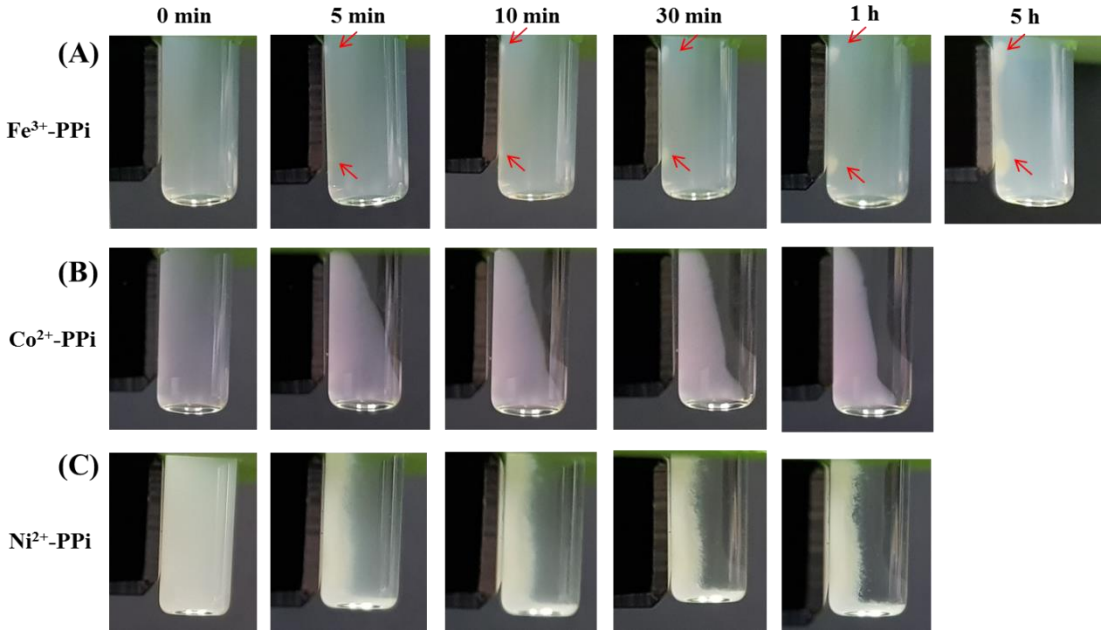

Figure S2. Examine of separation efficiency. Photographs of the samples (0.2 mL) containing PPi (0.01 M) and magnetic ions (0.025M) including (A) Fe^{3+}, (B) Co^{2+}, and (C) Ni^{2+} that were vortex-mixed for 30 min followed by magnetic separation. The time denoted on the top of the photographs indicated how long the magnet (the black bar adjacent to the left-hand side of each vial) had been placed next to the sample vial. The red arrows in Panel A indicated where the Fe^{3+}-PPi conjugates aggregated.

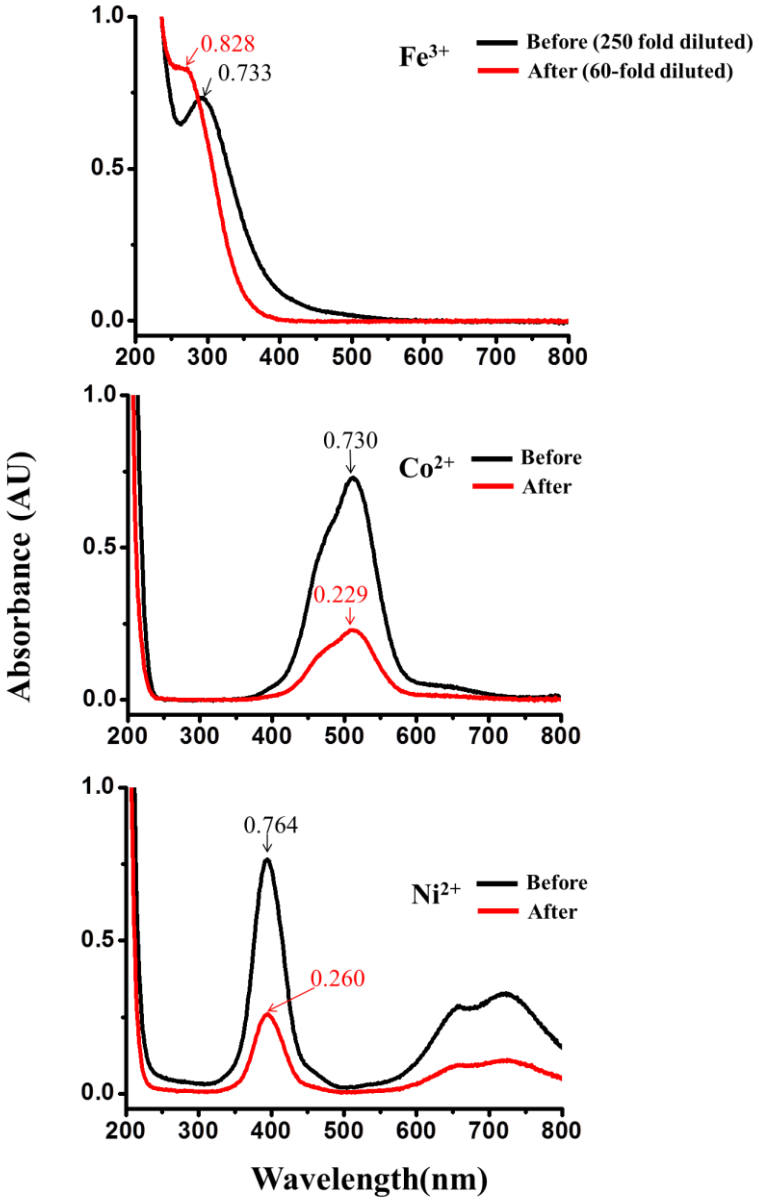

Figure S3. Examination of the ratio of magnetic ions to PPi in magnetic ion-PPi conjugates. UV-Vis absorption spectra of the supernatants obtained before (black band) and after (red band) vortex-mixing aqueous (A) $FeCl_3$ (0.1 M), (B) $CoCl_2$ (0.18 M), and (C) $NiCl_2$ (0.18 M) individually with PPi (0.05 M) followed by centrifugation at 10,000 rpm for 5 min. Owing to the high absorption coefficient of aqueous $FeCl_3$, aqueous $FeCl_3$ (0.1 M) and its supernatant obtained after precipitating with PPi were 250 and 60-fold diluted, respectively, prior to analysis by UV-Vis absorption spectroscopy.

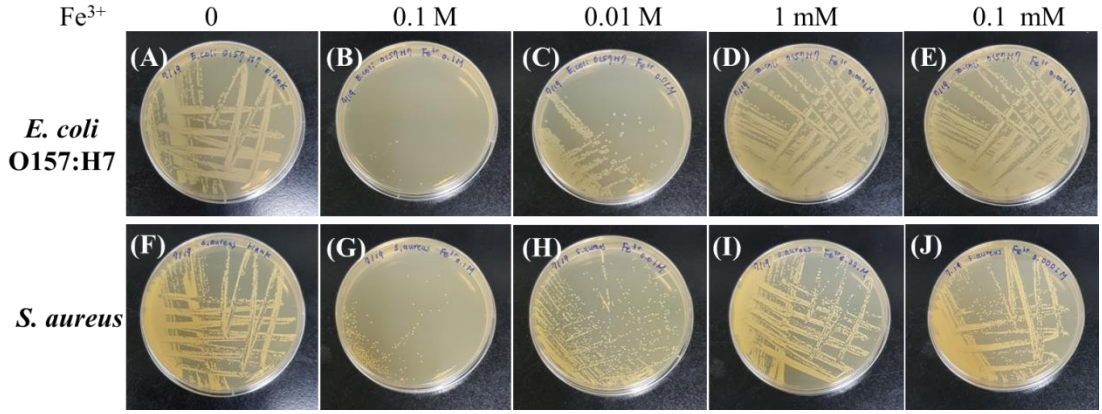

Figure S4. Examination of Fe^{3+} tolerance of bacteria. Photographs of the samples obtained by shaking *E. coli* O157:H7 (A-E) and *S. aureus* (F-J) individually with different concentrations of Fe^{3+} for 30 min followed by incubation on agar plates at 37 ^{o}C for overnight.

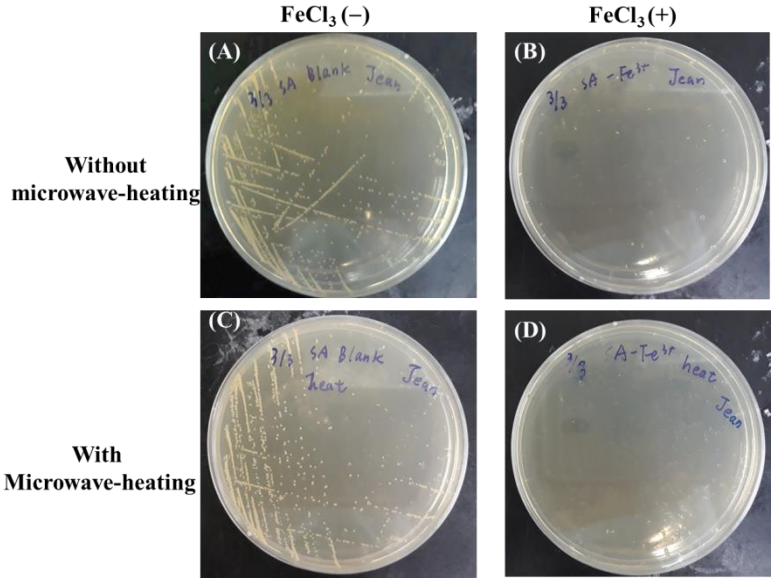

Figure S5. Examination of microwave-heating effects against bacteria. Photographs of the samples containing *S. aureus* (OD_{600}= ~1) in (A) the absence and (B) the presence of Fe^{3+} (0.1 M) by using an inoculation loop to take the sample (~6 μL) from the mixture for culture on agar plates at 37 ^{o}C for overnight. Photographs of the samples containing *S. aureus* (OD_{600}= ~1) in (C) the absence and (D) the presence of Fe^{3+} (0.1 M) under microwave-heating (power: 180 W) for three cycles (90 s $cycle^{-1}$) and using an inoculation loop to take the sample (~6 μL) from the mixture for culture on agar plates at 37 ^{o}C for overnight.

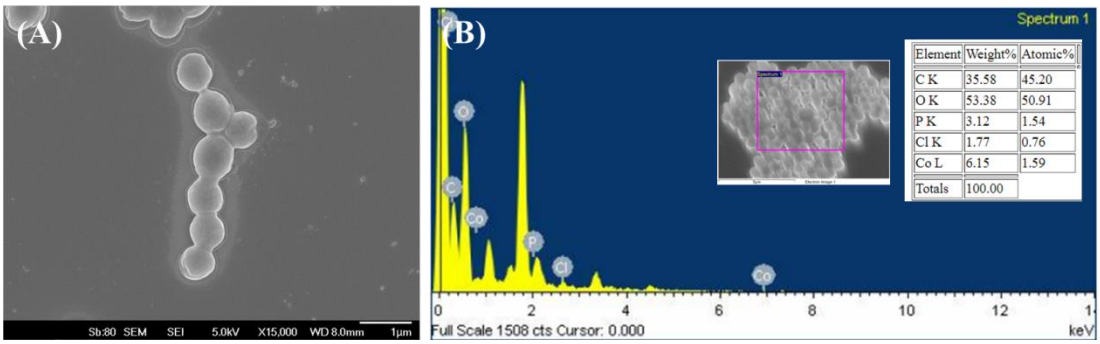

Figure S6. SEM image and EDX of Co^{2+}-*S. aureus* conjugates: (A) Representative SEM image of Co^{2+}-*S. aureus* conjugates. (B) EDX spectrum of the Co^{2+}-*S. aureus* conjugates. The inset SEM image shows where the EDX spectrum was obtained.

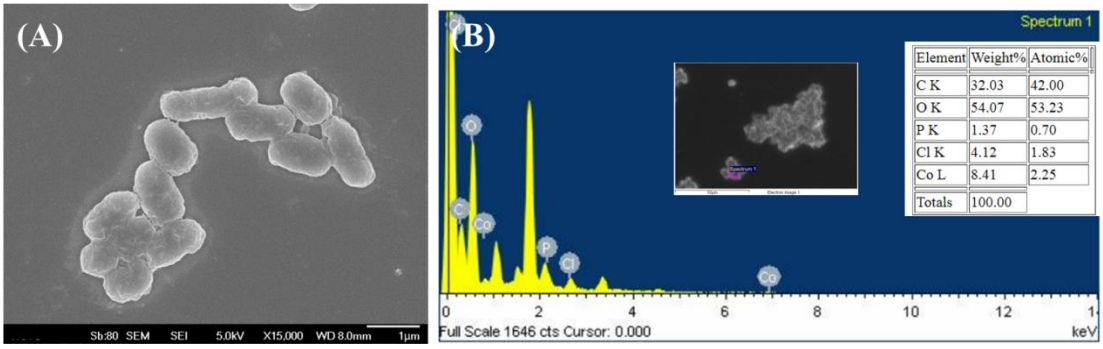

Figure S7. SEM image and EDX of Co^{2+}- *E. coli* O157:H7 conjugates: (A) Representative SEM image of Co^{2+}-*E. coli* O157:H7 conjugates. (B) EDX spectrum of the Co^{2+}-*E. coli* O157:H7 conjugates. The inset SEM image shows where the EDX spectrum was obtained.