Ferromagnetic nanoparticles with peroxidase-like activity enhance the cleavage of biological macromolecules for biofilm elimination

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Supplementary Figures

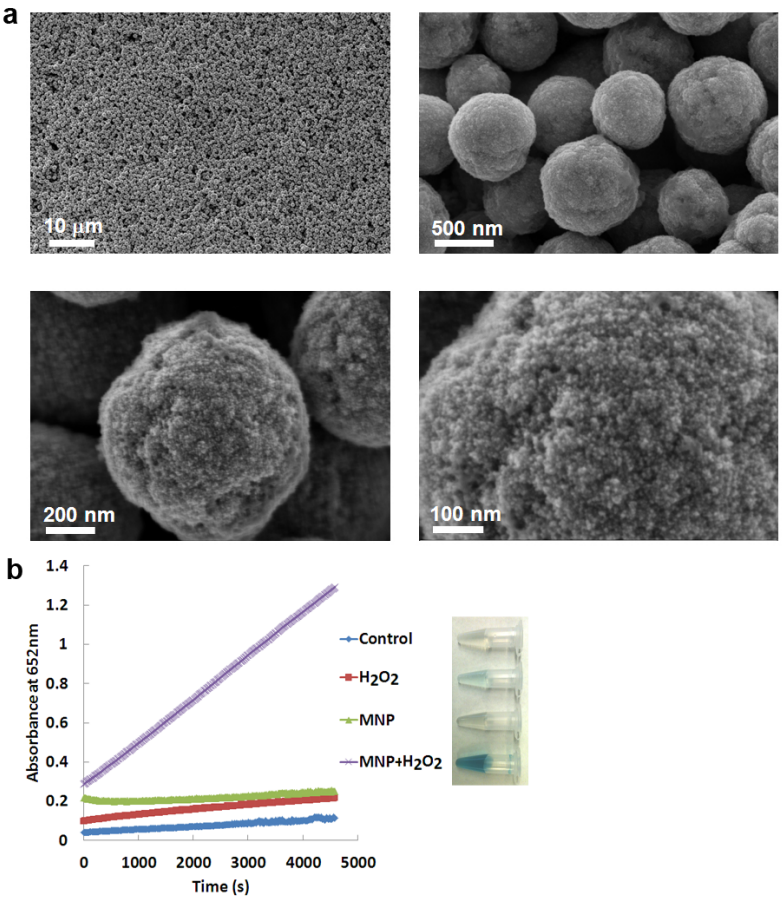

Figure S1. Characterization of MNP for morphology and activity. **a**, Characterization of MNP with scanning electron microscopy (SEM). All images are of the same nanoparticles but different magnifications. Single MNP had a diameter of approximately 500 nm. The rough surface of each particle was composed of small protrusions, each with a diameter of 5-10 nm. **b**, Peroxidase-like activity of MNP. The peroxidase-like activity was demonstrated using the TMB colorimetric reaction, comparing the effects of H_2O_2 and MNP alone and together. The presence of both MNP and H_2O_2 created the largest change in color (inserted small image) with absorbance measured at 652nm. This experiment was repeated in triplicate with a representative image shown.

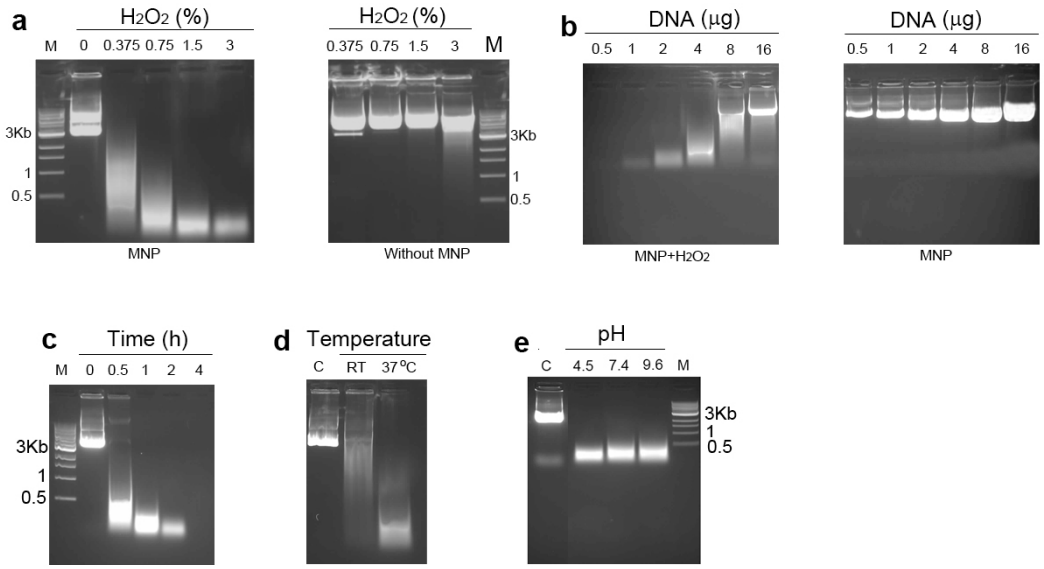

Figure S2. Plasmid DNA cleavage by MNP-H_2O_2. **a**, Effect of different H_2O_2 concentrations on cleavage with (**Left panel,** 20 μg of MNP**)**, or without (**Right panel**), the presence of MNP. Reaction components included 2 μg DNA in 50 μl NaAc (0.1M, pH 4.5), and incubations were for 1 hour at 37˚C. **b**, Effect of different concentrations of DNA on cleavage by MNP-H_2O_2 (**Left panel. 1% H_2O_2)**, or on plasmid DNA adsorption on MNP (**Right panel**). DNA was mixed with 20 μg of MNP in 50 μl NaAc (0.1M, pH 4.5) and incubated for 3 hour at 37˚C. **c**, Effect of time on cleavage of plasmid DNA by MNP-H_2O_2. 2 μg DNA was mixed with 20 μg of MNP and 1% H_2O_2 in 50 μl NaAc (0.1M, pH 4.5) and incubated at 37˚C for varying periods of time. **d**, Effect of reaction temperature on cleavage of plasmid DNA by MNP-H_2O_2. 2 μg DNA was mixed with 20 μg of MNP and 1% H_2O_2 in 50 μl NaAc (0.1M, pH 4.5) and incubated at 37˚C or room temperature (RT). **e**, Effect of pH on cleavage of plasmid DNA by MNP-H_2O_2. 2 μg DNA was mixed with 20 μg of MNP and 1% H_2O_2 in 50 μl NaAc (0.1M) with different pH and incubated for 1 hour at 37˚C. All DNA and cleavage products were identified by means of agarose gel electrophoresis and staining with ethidium bromide. In panels D and E, “C” denotes a control lane with DNA only. “M” denotes DNA marker. All experiments were repeated in triplicate with representative images shown.

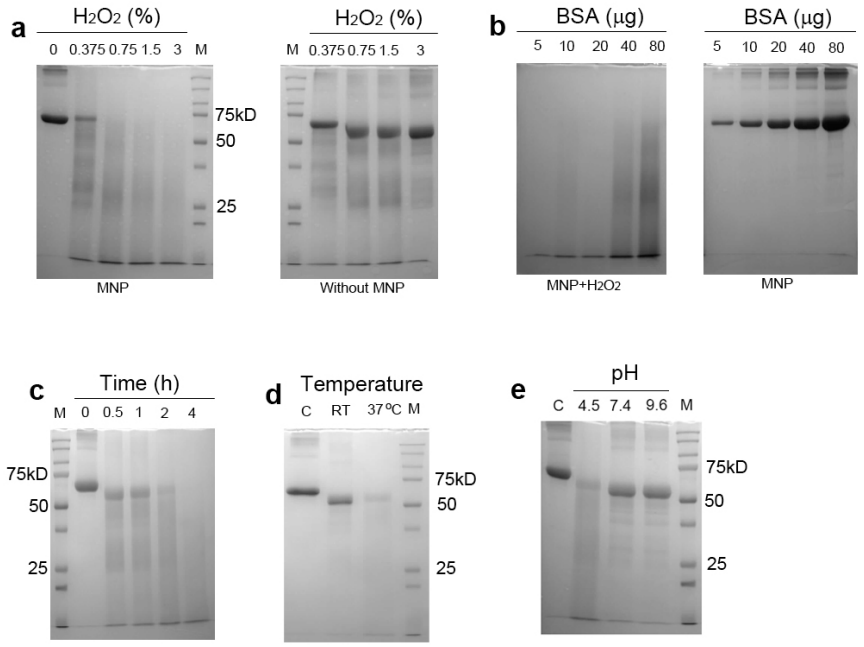

Figure S3. BSA cleavage by MNP-H_2O_2. **a**, Effect of different H_2O_2 concentrations on cleavage of BSA, with (**Left panel,** 20 μg of MNP**)**, or without (**Right panel**), the presence of MNP. Reaction components included 20 μg BSA in 50 μl NaAc (0.1M, pH 4.5), and incubations were for 3 hours at 37˚C. **b**, Effect of different concentrations of BSA on cleavage by MNP-H_2O_2 (**Left panel, 1%** H_2O_2**)**, or on BSA adsorption on MNP (**Right panel**). BSA was mixed with 20 μg of MNP in 50 μl NaAc (0.1M, pH 4.5) and incubated for 3 hour at 37˚C. **c**, Effect of time on cleavage of BSA by MNP-H_2O_2. 20 μg BSA was mixed with 20 μg of MNP and 1% H_2O_2 in 50 μl NaAc (0.1M, pH 4.5) and incubated at 37˚C for varying periods of time. **d**, Effect of reaction temperature on cleavage of BSA by MNP-H_2O_2. 20 μg BSA was mixed with 20 μg of MNP and 1% H_2O_2 in 50 μl NaAc (0.1M, pH 4.5) and incubated at 37˚C or room temperature (RT). **e**, Effect of pH on cleavage of BSA by MNP-H_2O_2. 20 μg BSA was mixed with 20 μg of MNP and 1% H_2O_2 in 50 μl NaAc (0.1M) with different pH and incubated for 1 hour at 37˚C. All full-length BSA and cleavage products were identified by means of SDS-PAGE and Coomassie staining. In panels D and E, "C" denotes a control lane with BSA only. "M" denotes protein marker. All experiments were repeated in triplicate with representative images shown.

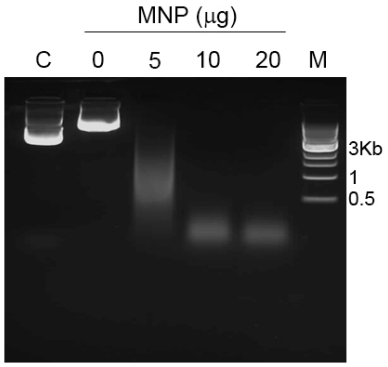

Figure S4. Plasmid DNA cleavage by MNP-H_2O_2 with different concentrations of MNP. 2 μg DNA was mixed with 1% H_2O_2 in 50 μl NaAc (0.1M, pH 4.5) and incubated for 3 hour at 37˚C. "C" denotes a control lane with DNA only. "M" denotes DNA marker. DNA and cleavage products were identified by means of agarose gel electrophoresis and staining with ethidium bromide. The experiment was repeated in triplicate with a representative image shown.

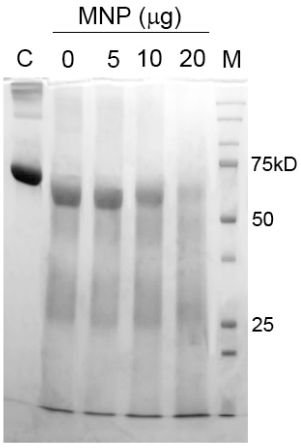

Figure S5. BSA cleavage by MNP-H_2O_2 with different concentrations of MNP. 20 μg BSA was mixed with 1% H_2O_2 in 50 μl NaAc (0.1M, pH 4.5) and incubated for 3 hour at 37˚C. "C" denotes a control lane with BSA only. "M" denotes protein marker. All full-length BSA and cleavage products were identified by means of SDS-PAGE and Coomassie staining. The experiment was repeated in triplicate with a representative image shown.

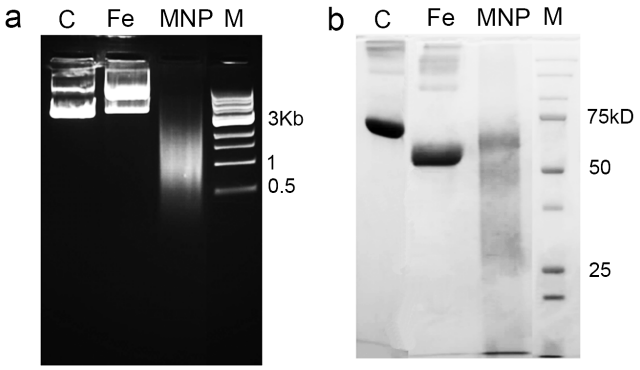

Figure S6. Cleavage with MNP versus released Fe ions. 20 µg MNP were incubated in 50 µl NaAc (0.1M, pH 4.5) for 1 hour at 37˚C, and then collected by centrifugation. The supernatant and resuspended MNP were separately mixed with 1% H_2O_2 and either 2µg DNA (a) or 20 µg BSA (b) and incubated to see which was responsible for cleavage. "M" denotes DNA or protein marker, respectively. "C" denotes control with DNA or BSA only. All experiments were repeated in triplicate with representative images shown.

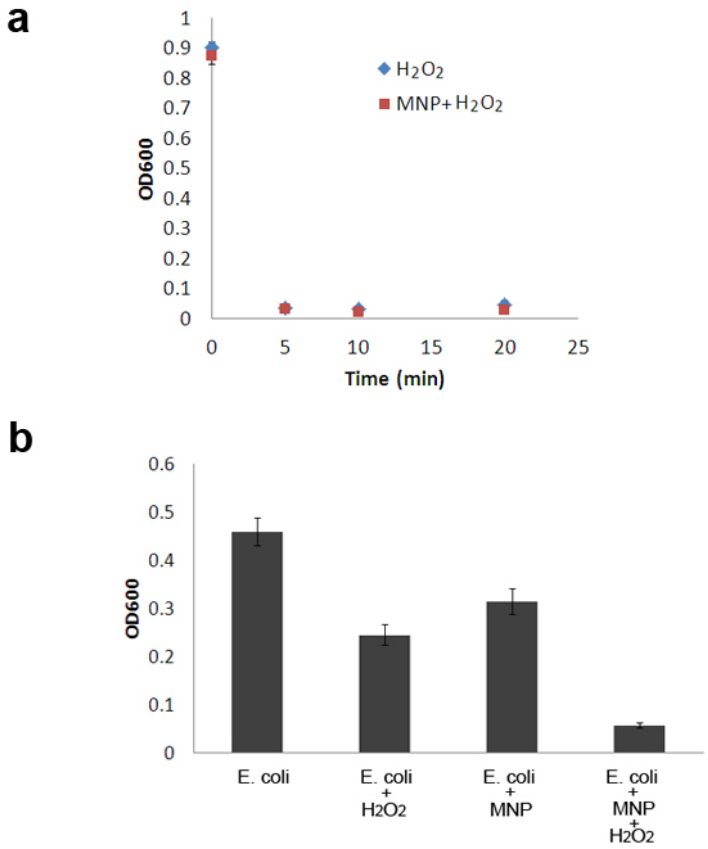

Figure S7. Killing of *E. coli* by H_2O_2 alone or with MNP. **a**, Effect of 1% H_2O_2 alone versus MNP-H_2O_2 on bacteria in suspension as measured by optical density. TOP10 cells were cultured in LB with ampicillin (100 µg/ml) in liquid media at 37˚C overnight. Bacterial count was reflected by an OD600 of approximately 2.0. The cells were spun down and 10 µl of the pellet were resuspended in 50 µl NaAC (0.1 M, pH 4.5) containing 1% H_2O_2 alone or 20 µg of MNP with 1% H_2O_2. 5 µl of each mixture were transferred into 1 ml LB liquid media containing ampicillin and incubated for 6 hours to detect OD600. **b,** Enhanced killing of *E. coli* by MNP-H_2O_2 with low dose, 0.01% H_2O_2. TOP10 cells were cultured in LB with ampicillin (100 µg/ml) in liquid media at 37˚C overnight. Bacterial count was reflected by an OD600 of approximately 2.0. The cells were spun down and 2 µl of the pellet were resuspended in either 50 µl NaAC (0.1 M, pH 4.5), or this medium containing 0.01% H_2O_2 alone, or this medium containing 20 µg of MNP, or this medium containing 20 µg of MNP with 0.01% H_2O_2. The half of each suspension (25 µl) was transferred into 1 ml LB containing ampicillin (100 µg/ml) liquid medium. The OD600 was detected after incubation at 37˚C for 6 hours, reflecting the cell growth of TOP10 *E.coli* after treatment with the different conditions. When individually compared against the control, H_2O_2, MNP alone, and MNP- H_2O_2 were found to be significantly different ($p<0.05$, n=3, Student's T test). When compared against each other, the OD600 remaining after treatment with MNP- H_2O_2 was significantly lower than when treated with H_2O_2 alone or MNP alone ($p<0.05$, n=3, Student's T test). Error bars denote standard deviation.

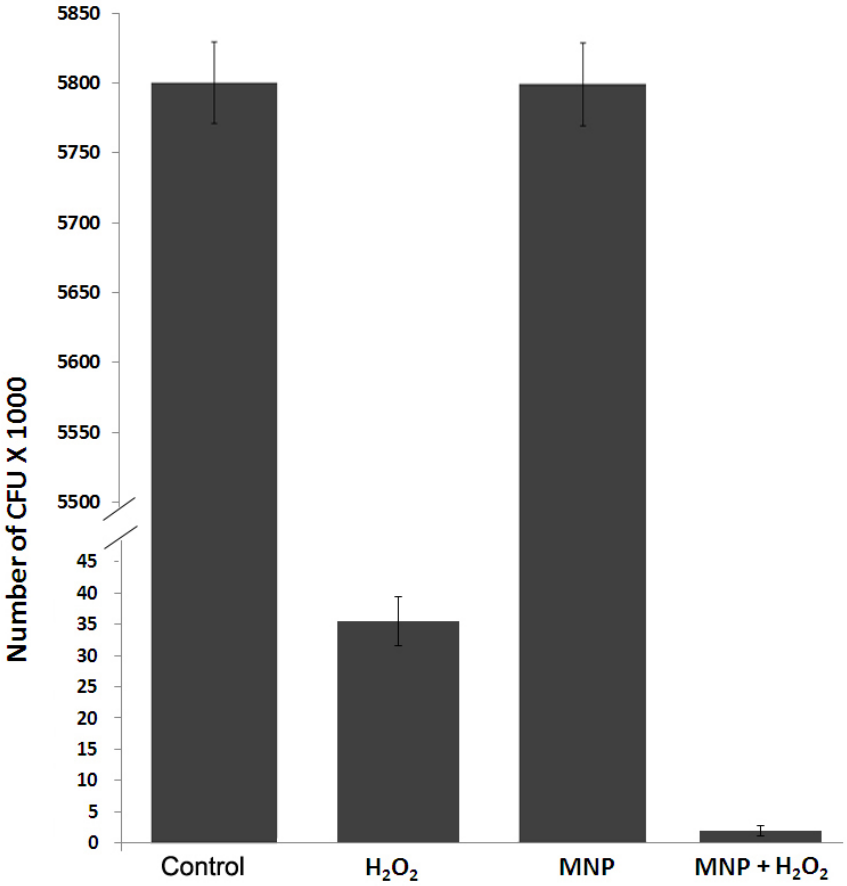

Figure S8. Viability of *Pseudomonas aeruginosa* collected from biofilms treated with H_2O_2, MNP, MNP-H_2O_2, or without any treatment (Control). Biofilms were challenged with minimal media supplemented with 1% H_2O_2, or minimal media supplemented with 1% H_2O_2 + MNP. After removing liquid medium from the wells, a sterile cotton swab was used to remove biofilm and resident cells from each well and the swab was subsequently used to inoculate 500 µL of M63 salts. Dilutions were made and 100 µL aliquots of each dilution were plated onto LB agar plates, and incubated overnight at 37°C. Colonies were counted and the number of colony forming units (CFU) was compared for each sample. When compared against each other, the number of CFU remaining after treatment with MNP-H_2O_2 was significantly lower than when treated with H_2O_2 alone ($p<0.05$, n=3, Student's T test). Error bars denote standard deviation. Note that the Y axis is discontinuous to convey the number of CFU under the different conditions.