

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

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Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is a “green chemical” that has various cleaning and disinfectant uses, including as an anti-bacterial agent for hygienic and medical treatments. However, its efficacy is limited against biofilm-producing bacteria, because of poor penetration into the protective, organic matrix. Here we show new applications for ferromagnetic nanoparticles (Fe<sub>3</sub>O<sub>4</sub>, MNPs) with peroxidase-like activity in potentiating the efficacy of H<sub>2</sub>O<sub>2</sub> in biofilm degradation and prevention. Our data show that MNPs enhanced oxidative cleavage of biofilm components (model nucleic acids, proteins, and oligosaccharides) in the presence of H<sub>2</sub>O<sub>2</sub>. When challenged with live, biofilm-producing bacteria, the MNP–H<sub>2</sub>O<sub>2</sub> system efficiently broke down the existing biofilm and prevented new biofilms from forming, killing both planktonic bacteria and those within the biofilm. By enhancing oxidative cleavage of various substrates, the MNP–H<sub>2</sub>O<sub>2</sub> system provides a novel strategy for biofilm elimination, and other applications utilizing oxidative breakdown.

H<sub>2</sub>O<sub>2</sub> has been used as a general anti-bacterial agent for hygienic and medical treatments. H<sub>2</sub>O<sub>2</sub> generates free radicals which oxidize organic chemicals or biomolecules, but the process is slow with low efficiency and bacteria easily develop resistance,<sup>1,2</sup> especially in a formed biofilm. Biofilms represent an enormous medical challenge, responsible for millions of healthcare-associated infections annually worldwide.<sup>3–6</sup> Biofilms consist of cells within a matrix of nucleic acids, proteins and polysaccharides, which connect and protect resident bacteria from external damage.<sup>7–9</sup> Nanocatalysts that could potentiate the effects of H<sub>2</sub>O<sub>2</sub> might have great utility for a

variety of applications,<sup>10–14</sup> potentially including biofilm destruction in the sterilization of medical surfaces, surgical instruments, and indwelling medical devices.

Recently, it has been reported that MNPs possess an intrinsic peroxidase-like catalytic activity, which can effectively generate free radicals from H<sub>2</sub>O<sub>2</sub> with high efficiency, similar to horseradish peroxidase (HRP).<sup>10,15</sup> MNPs are promising nanocatalysts because of their magnetic properties, high catalytic activity, and adjustability through nanoscale modifications,<sup>10,16–19</sup> suggesting possible uses in immunoassays,<sup>10,13,20,21</sup> organic pollutant degradation,<sup>12,22–26</sup> glucose detection<sup>17,18,27–31</sup> and cancer diagnostics.<sup>14</sup> Although it has been reported that MNPs alone or combined with H<sub>2</sub>O<sub>2</sub> can prevent biofilm formation by inhibiting bacterial growth,<sup>32,33</sup> there are no reports regarding the efficacy of an MNP–H<sub>2</sub>O<sub>2</sub> system on biofilm destruction and killing of bacteria resident within biofilms—a much more difficult medical challenge.

## Results and discussion

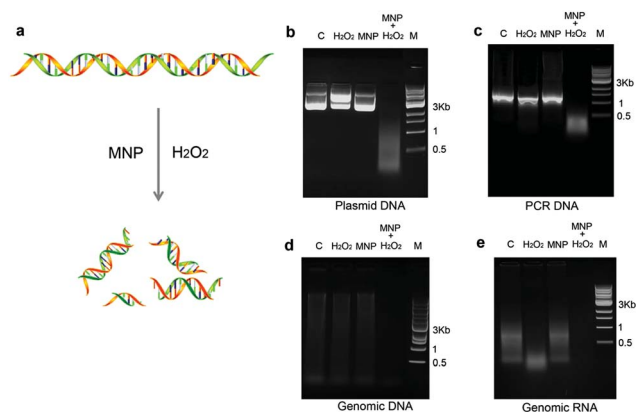
To test the ability of an MNP–H<sub>2</sub>O<sub>2</sub> system to degrade biofilms and kill resident bacteria, we hydrothermally prepared MNPs having a bulk morphological diameter of 500 nm, with a rough surface containing 5–10 nm diameter protrusions (Fig. S1a†). These nanoparticles show very high peroxidase-like activity as demonstrated by the 3,3',5,5'-tetramethylbenzidine (TMB) colorimetric reaction (Fig. S1b†). We then used these MNPs to test whether the MNP–H<sub>2</sub>O<sub>2</sub> system could degrade each of the 3 major components of biofilms. We used H<sub>2</sub>O<sub>2</sub> at concentrations of 1–3%, consistent with domestic hygiene uses, to investigate first the MNP–H<sub>2</sub>O<sub>2</sub> system's ability to degrade nucleic acids. Long chain plasmid DNA was completely cleaved into small fragments (Fig. 1b). Plasmid DNA in the presence of H<sub>2</sub>O<sub>2</sub> alone showed topological change from supercoiled structure to linearized form but catalysis into fragments was dependent upon the presence of MNPs (Fig. S2a†). Cleavage of plasmid DNA was also dependent upon concentrations of H<sub>2</sub>O<sub>2</sub> and DNA, as well as the time and temperature (Fig. S2a–d†), but was not

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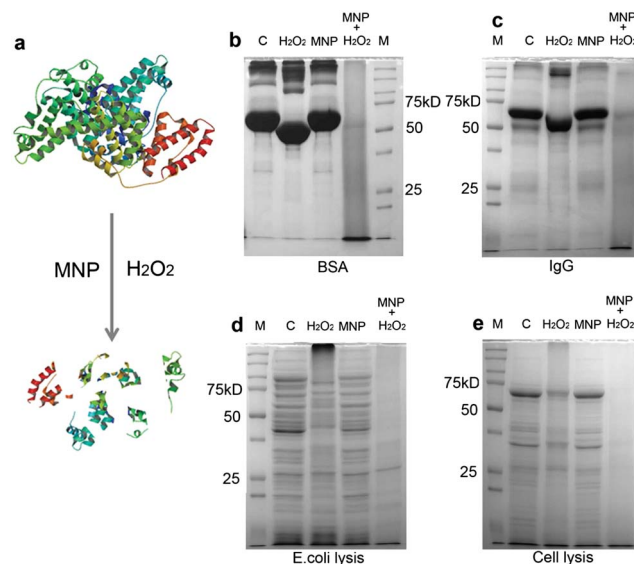


**Fig. 1** MNP enhanced oxidative cleavage of nucleic acids. (a) Schematic of MNP-H<sub>2</sub>O<sub>2</sub> cleaving nucleic acids. (b) Plasmid DNA cleavage with MNP-H<sub>2</sub>O<sub>2</sub>. Plasmid DNA (4 µg, ≈ 6500 bp) was incubated with 3% H<sub>2</sub>O<sub>2</sub> alone, MNP alone, or MNP-H<sub>2</sub>O<sub>2</sub>. "C" denotes control with DNA only. "M" denotes the DNA marker (1 kb DNA ladder, NEB). (c) Cleavage processes of a PCR DNA product (1 µg), (d) mouse genomic DNA (1 µg), and (e) mouse total RNA (from testis) were all performed under similar conditions but with 1% H<sub>2</sub>O<sub>2</sub>. All experiments were repeated in triplicate with representative images shown.

influenced by the pH within the tested range of 4.5–9 (Fig. S2e†). The only slight difference among the various forms of nucleic acids we tested was that total RNA showed slight cleavage by H<sub>2</sub>O<sub>2</sub> alone (Fig. 1e). Our results indicated that the MNP-H<sub>2</sub>O<sub>2</sub> system could be used as a universal reagent for cleaving or degrading nucleic acids, suggesting additional applications for this system beyond biofilm degradation.

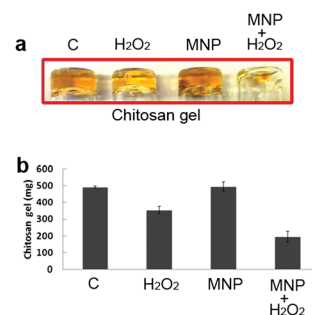
We next investigated the cleavage of proteins by the MNP-H<sub>2</sub>O<sub>2</sub> system using similar conditions as above (Fig. 2a). In our first trial, we found that BSA (80 µg, MW = 66 kDa) was cleaved into small fragments as seen in a SDS-PAGE gel stained with Coomassie blue R-250. In contrast, BSA treated with 3% H<sub>2</sub>O<sub>2</sub> alone showed very limited cleavage (Fig. 2b). We next varied the concentrations of H<sub>2</sub>O<sub>2</sub> or BSA while keeping the reaction buffer constant (Fig. S3†). Although higher concentrations of H<sub>2</sub>O<sub>2</sub> led to some cleavage, complete cleavage was dependent upon the presence of MNPs (Fig. S3a†). Lowering the amount of protein, or increasing the time of reaction or the temperature from room temperature to 37 °C resulted in more cleavage as expected (Fig. S3b–d†). However, unlike the effect on DNA cleavage, protein cleavage by the MNP-H<sub>2</sub>O<sub>2</sub> system showed obvious pH dependence with increased cleavage at lower pH (Fig. S3e†). We assessed the versatility of the cleavage using more complex proteins, including IgG and lysates from whole bacteria and mammalian cells. We found that cleavage was not protein specific; mixtures of the wide variety of proteins found in cell lysates were completely cleaved under the same reaction conditions, demonstrating the generality of the cleavage mediated by the MNP-H<sub>2</sub>O<sub>2</sub> system.

Polysaccharides are the third major component in the biofilm matrix.<sup>34</sup> We chose chitosan as a model polysaccharide to test the effect of MNP-H<sub>2</sub>O<sub>2</sub> versus H<sub>2</sub>O<sub>2</sub> alone. We prepared a chitosan hydrogel by glutaraldehyde crosslinking in a glass vial. The chitosan gel had a yellow color with high adherence and



**Fig. 2** MNP enhanced oxidative cleavage of proteins. (a) Schematic of MNP-H<sub>2</sub>O<sub>2</sub> cleaving protein. (b) Cleavage of BSA with MNP-H<sub>2</sub>O<sub>2</sub>. 80 µg BSA was incubated with 3% H<sub>2</sub>O<sub>2</sub> alone, MNP alone, or MNP-H<sub>2</sub>O<sub>2</sub>. "C" denotes control with BSA only. "M" denotes the protein molecular weight marker (Precision Plus Protein™ Dual Color Standards, BIO-RAD). (c) Cleavage processes of rabbit polyclonal IgG (10 µg), (d) *E. coli* lysate (40 µg), and (e) HEK293 mammalian cell lysate (20 µg) were all performed under similar conditions but with 1% H<sub>2</sub>O<sub>2</sub>. All experiments were repeated in triplicate with representative images shown.

viscosity, enabling it to stay suspended upside down in a tube (Fig. 3a). Although H<sub>2</sub>O<sub>2</sub> alone cleaved the crosslinked chitosan to a degree, MNP-H<sub>2</sub>O<sub>2</sub> had a greater effect at reducing the mass that remained as a gel (Fig. 3). We also found that chitosan cleavage was independent of pH, with cleavage occurring under both acidic (pH 4.5) and neutral (pH 7.4) conditions (data not shown).



**Fig. 3** MNP enhanced cleavage of a polysaccharide (chitosan). A chitosan gel was prepared at 0.5% with glutaraldehyde crosslinking. Chitosan gels were mixed with 1% H<sub>2</sub>O<sub>2</sub> alone, MNP alone, or MNP-H<sub>2</sub>O<sub>2</sub> to test for cleavage. "C" denotes control with chitosan only. All experiments were repeated in triplicate with representative images shown. When individually compared against the control, H<sub>2</sub>O<sub>2</sub> and MNP-H<sub>2</sub>O<sub>2</sub> were found to be significantly different ( $p < 0.05$ ,  $n = 3$ , Student's  $t$ -test). When compared against each other, the amount of biofilm remaining after treatment with MNP-H<sub>2</sub>O<sub>2</sub> was significantly less than that when treated with H<sub>2</sub>O<sub>2</sub> alone ( $p < 0.05$ ,  $n = 3$ , Student's  $t$ -test). Error bars denote the standard deviation.

We next sought to verify the mechanism by which the MNP-H<sub>2</sub>O<sub>2</sub> system was able to enhance cleavage of nucleic acids, proteins, and polysaccharides, hypothesizing that the benefits were obtained through production of additional free radical oxidants generated by MNP catalysis of H<sub>2</sub>O<sub>2</sub>. To test the hypothesis, we investigated the impacts on cleavage of an antioxidant reagent, hypotaurine, which specifically scavenges hydroxyl radicals. Fig. 4a shows plasmid DNA cleavage in the presence of varied concentrations of hypotaurine (1–8%). We observed that cleavage was retarded with increasing concentrations of hypotaurine until it was almost stopped with 8% hypotaurine. Fig. 4b shows a similar reduction in cleavage of BSA. These data suggested that enhanced cleavage was dependent on free hydroxyl radicals produced by MNPs with peroxidase-like activity (Fig. 4c).

Therefore, it should be possible to control the kinetics or extent of cleavage by adjusting the generation of these free radical oxidants. A simple way to do this would be to change the amount of the catalyst, MNPs. We varied the amounts of MNPs (0–20 μg) while keeping the other components constant in the cleavage reaction and found that 10–20 μg of MNPs cleaved 2 μg of plasmid DNA into small pieces below 100 bp, but 5 μg of MNPs resulted in fragments between 500 bp and 1000 bp (Fig. S4†). The effects on cleavage of BSA were similar in that 5 μg of MNPs only moderately reduced the intensity of the full-length band, with increasing cleavage by 10 or 20 μg MNPs (Fig. S5†).

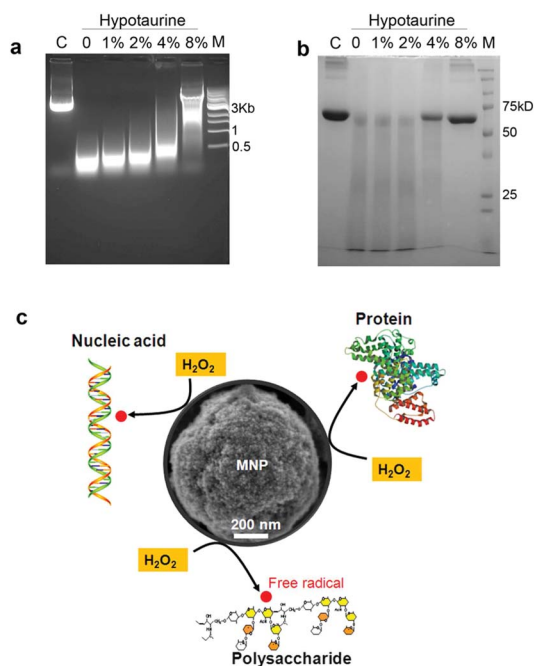


Fig. 4 Mechanism of MNP-enhanced oxidative cleavage. The antioxidant, hypotaurine, inhibited cleavage of 2 μg plasmid DNA (a) or 20 μg BSA (b) by MNP-H<sub>2</sub>O<sub>2</sub> in a dose-dependent fashion. "M" denotes the DNA or protein marker, respectively. "C" denotes control with DNA or BSA only. All experiments were repeated in triplicate with representative images shown. (c) Schematic of MNP-enhanced cleavage of a nucleic acid, a protein and a polysaccharide. MNPs catalyze H<sub>2</sub>O<sub>2</sub> with high efficiency, making free radicals that attack these biomolecules.

To this point, our data were consistent with the Fenton reaction, in which iron and H<sub>2</sub>O<sub>2</sub> interact, yielding radical oxidants. We wished to determine whether cleavage was initiated from the nanoparticles, or from the supernatant which might have leached free Fe ions. To investigate this, we pre-incubated the MNPs in the NaAc buffer (0.1 M, pH 4.5, 1 h) without adding H<sub>2</sub>O<sub>2</sub> or DNA/BSA. We then collected the MNPs by centrifugation, removed the supernatant, and resuspended the MNPs in the same buffer. We separately conducted cleavage reactions as above by adding H<sub>2</sub>O<sub>2</sub> and plasmid DNA or BSA to both the supernatant and the resuspended MNPs. We observed significantly higher cleavage for both DNA and BSA in the tubes with the MNPs (Fig. S6a and b†), suggesting that the MNPs were primarily responsible for cleavage, which is consistent with our previous research and the findings of others under similar experimental conditions.<sup>10,16,27</sup>

Having demonstrated an ability to enhance cleavage of the individual components of biofilms, we next sought to test the effects of the MNP-H<sub>2</sub>O<sub>2</sub> system on live bacteria. When *E. coli* were plated, 1% H<sub>2</sub>O<sub>2</sub> in 50 μl NaAc buffer (0.1 M, pH 4.5) killed 98% of the bacteria within 5 min, in the presence or absence of MNPs (Fig. S7a†). However, we observed equivalent killing when incubated with 0.01% H<sub>2</sub>O<sub>2</sub> in the presence of 20 μg MNPs, confirming enhanced bacterial killing by the MNP-H<sub>2</sub>O<sub>2</sub> (Fig. S7b†). This result was consistent with prior studies using MNPs for anti-bacterial properties including prevention of biofilm formation.<sup>32</sup>

As *E. coli* die, they release a complex mixture of cellular components including nucleic acids and proteins. The released biomass might accumulate around resistant cells and, therefore, contribute to biofilm formation and protection from disinfectants or antimicrobial treatments. To test whether the MNP-H<sub>2</sub>O<sub>2</sub> system would degrade these complex mixtures, we collected the released nucleic acids and proteins and incubated them with MNP-H<sub>2</sub>O<sub>2</sub> or controls, including either MNPs or H<sub>2</sub>O<sub>2</sub>. Fig. 5 shows that treatment with MNP-H<sub>2</sub>O<sub>2</sub> was successful at degrading these released products, thus not only efficiently killing bacteria, but also degrading the biomass released from the dead cells.

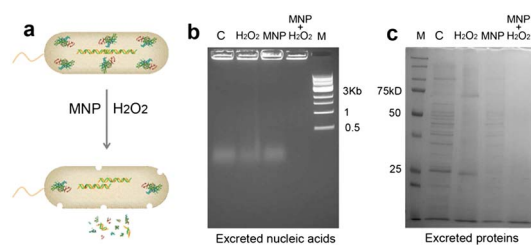
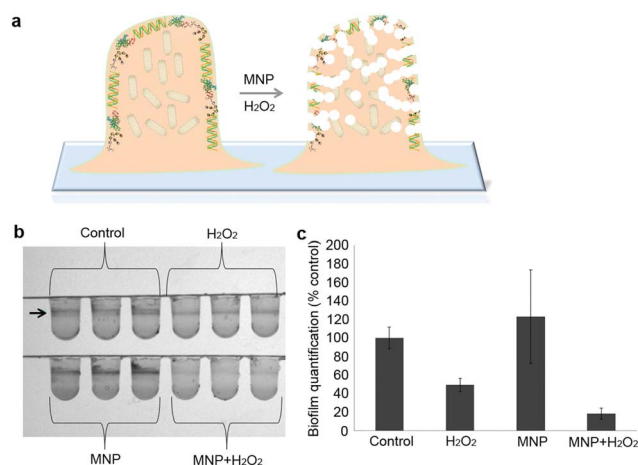


Fig. 5 Killing of *E. coli* and cleavage of released nucleic acids and proteins by the MNP-H<sub>2</sub>O<sub>2</sub> system. (a) Schematic of MNP-H<sub>2</sub>O<sub>2</sub> killing of *E. coli* and cleavage of released nucleic acids and proteins. (b) MNP-H<sub>2</sub>O<sub>2</sub> cleaved nucleic acids released from killed *E. coli* cells. "C" denotes control with nucleic acids only. "M" denotes the DNA marker. (c) MNP-H<sub>2</sub>O<sub>2</sub> cleaved proteins released from killed *E. coli* cells. "M" denotes the protein marker. These experiments show that the MNP-H<sub>2</sub>O<sub>2</sub> system was able to degrade the complex mixture of organic components that results from bacterial death. Penetration of organic materials would be an important characteristic of a disinfectant. All experiments were repeated in triplicate with representative images shown.

These data led us to study whether the MNP-H<sub>2</sub>O<sub>2</sub> system could penetrate and eliminate biofilms, facilitating killing of resident bacteria as well as planktonic bacteria. We addressed these questions using a biofilm from *Pseudomonas aeruginosa*, a common cause of nosocomial infection and often resistant to treatment due to the biofilm. We allowed the bacteria to form biofilms in 96-well plates in advance of treatment with MNP-H<sub>2</sub>O<sub>2</sub>, and then observed and quantified remaining biofilms with 0.1% crystal violet. Treatment with MNP-H<sub>2</sub>O<sub>2</sub> in either NaAc buffer (0.1 M, pH 4.5, 1% H<sub>2</sub>O<sub>2</sub>, for 2 h) or biofilm minimal media (M63 medium, pH ≈ 7) both resulted in obvious reduction in remaining biofilms (Fig. 6, 18 ± 6.17% of the biofilm remained after treatment). In contrast, wells treated with either H<sub>2</sub>O<sub>2</sub> or MNP alone still showed a clear biofilm band, with 49 ± 7.37% of the biofilm remaining after treatment with H<sub>2</sub>O<sub>2</sub> alone. The difference demonstrated that MNP-H<sub>2</sub>O<sub>2</sub> eliminated the formed biofilm with much higher efficiency than H<sub>2</sub>O<sub>2</sub>. We next assessed the viability of bacteria resident within the biofilm, quantifying the number of colony forming units (CFUs) of bacteria recovered from the untreated biofilm (control) versus MNP alone, versus H<sub>2</sub>O<sub>2</sub> alone, and versus MNP-H<sub>2</sub>O<sub>2</sub>. The MNP alone-treated biofilm had almost no change in CFUs versus the control (Fig. S8†). Incubation with H<sub>2</sub>O<sub>2</sub> alone reduced CFUs by approximately 2 orders of magnitude, whereas MNP-H<sub>2</sub>O<sub>2</sub> reduced CFUs by an additional factor of 20 (Fig. S8†).



**Fig. 6** Biofilm elimination by MNP-enhanced oxidative cleavage. (a) Schematic of MNP-H<sub>2</sub>O<sub>2</sub> eliminating the biofilm by cleaving nucleic acids, proteins and polysaccharides in the biofilm. (b) *Pseudomonas aeruginosa* biofilm elimination with MNP-H<sub>2</sub>O<sub>2</sub>. The formed biofilm (remaining planktonic cells were removed from wells) was treated with MNP-H<sub>2</sub>O<sub>2</sub> and stained with crystal violet dye (arrow points to the ring of the biofilm). This experiment was repeated in triplicate with a representative image shown. (c) Quantification of the biofilm remaining after MNP-H<sub>2</sub>O<sub>2</sub> treatment. When individually compared against the control, H<sub>2</sub>O<sub>2</sub> and MNP-H<sub>2</sub>O<sub>2</sub> were found to be significantly different ( $p < 0.05$ ,  $n = 3$ , Student's  $t$ -test). When compared against each other, the amount of biofilm remaining after treatment with MNP-H<sub>2</sub>O<sub>2</sub> was significantly less than that when treated with H<sub>2</sub>O<sub>2</sub> alone ( $p < 0.05$ ,  $n = 3$ , Student's  $t$ -test). Error bars denote the standard deviation.

## Conclusions

The combined results of our *in vitro* assays and biofilm quantification and cell viability assays suggested that the MNP-H<sub>2</sub>O<sub>2</sub> system had marked advantages over the use of H<sub>2</sub>O<sub>2</sub> alone. In particular, the intrinsic peroxidase-like activity of MNPs resulted in the enhanced cleavage of nucleic acids, proteins and polysaccharides. The new features could be used for the degradation of and penetration into the formed biofilm, as well as the killing of planktonic bacteria and prevention of biofilm formation. Enhanced oxidative cleavage by the MNP-H<sub>2</sub>O<sub>2</sub> system therefore provides a promising new approach for cleaning of medical surfaces and instruments that are often resistant to disinfection because of biofilms.

## Materials and methods

Sodium acetate (NaAc), ethanol, and Coomassie blue R-250 were purchased from Fisher Scientific. Ethylene glycol was purchased from J.T. Baker. Iron(III) chloride (FeCl<sub>3</sub>), bovine serum albumin (BSA), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (30% stock), chitosan (low molecular weight), ethidium bromide (EB), hypotaurine, HRP, 3,3',5,5'-tetramethylbenzidine (TMB), mouse IgG, and anti-mouse IgG were purchased from Sigma-Aldrich (St. Louis, MO, USA). Plasmid DNA, testis RNA, and lysates from *E. coli* and HEK293 cells were prepared in our laboratory in the presence of protease inhibitors (Protease Inhibitor Cocktail Tablets, Roche Applied Science) using standard approaches.

### Preparation of ferromagnetic nanoparticles with peroxidase-like activity

Ferromagnetic nanoparticles (MNPs) were prepared and used as catalysts in these experiments. Fe<sub>3</sub>O<sub>4</sub> MNPs were synthesized in one-step in a solvothermal system by combining FeCl<sub>3</sub> and NaAc in ethylene glycol. Briefly, 0.82 g of FeCl<sub>3</sub> was dissolved in 40 ml of ethylene glycol to form a clear solution. Then, 3.6 g of NaAc was added to the solution with vigorous stirring for 30 min. The mixture was then transferred to a 50 ml teflon-lined stainless-steel autoclave and left to react at 200 °C for 12 h. After the autoclave cooled to room temperature, the black precipitate was collected, rinsed several times using ethanol and then dried at 60 °C. The synthesized nanoparticles were characterized using scanning electron microscopy (SEM; Philips XL-30 field, 15 kV). The peroxidase-like activity was tested in a mixture of 500 μl NaAc buffer (0.1 M, pH 4.5) containing 20 μg MNPs, 0.3% H<sub>2</sub>O<sub>2</sub> and 100 μg TMB. The blue color produced was recorded with a spectrophotometer at 652 nm.

### Cleavage of nucleic acids and proteins

Unless indicated otherwise, nucleic acid cleavage assays were performed at 37 °C for 3 hours in 50 μl NaAc buffer (0.1 M, pH 4.5) containing 20 μg MNPs and 1–3% H<sub>2</sub>O<sub>2</sub>. In these and later experiments, nucleic acid cleavage products were identified by agarose gel electrophoresis and ethidium bromide staining. Protein cleavage assays were performed at 37 °C for 3 hours in 50 μl NaAc buffer (0.1 M, pH 4.5) containing 20 μg MNPs

and 1–3% H<sub>2</sub>O<sub>2</sub> prior to separation by SDS-PAGE and Coomassie staining.

### Cleavage of polysaccharides

A chitosan hydrogel was prepared by glutaraldehyde cross-linking. Briefly, chitosan at 0.5% was dissolved in 500  $\mu$ l NaAc (0.1 M, pH 4.5) and incubated with 0.2% glutaraldehyde at 37 °C for 1 hour. The formed hydrogel had a yellow color in a glass vial and its weight was recorded as “before cleavage”. 120  $\mu$ g of MNPs or 120  $\mu$ g of MNPs with 1% H<sub>2</sub>O<sub>2</sub> in 300  $\mu$ l NaAc (0.1 M, pH 4.5) was added to cover the gel in the glass vial and incubated at 37 °C for 1 hour. Then the supernatant was discarded and the remaining gel was rinsed 3 times with water. Exposure to filter paper for 1 hour was used to remove water and uncrosslinked chitosan, and the mass of the remaining gel was recorded as “after cleavage”. The volume of the gel remaining in the glass vial was visualized after inversion.

### Hypotaurine inhibition of cleavage with MNPs

To investigate the effect of hypotaurine on cleavage by MNP–H<sub>2</sub>O<sub>2</sub>, we incubated either 2  $\mu$ g plasmid DNA or 20  $\mu$ g of BSA at 37 °C for 3 hours in 50  $\mu$ l NaAc buffer (0.1 M, pH 4.5) containing varying concentrations of hypotaurine (1–8%), with 20  $\mu$ g MNPs and 1% H<sub>2</sub>O<sub>2</sub>. To investigate the relative catalytic effects of leached Fe ions *versus* the MNPs, we incubated 20  $\mu$ g MNPs in 50  $\mu$ l NaAc (0.1 M, pH 4.5) for 1 hour at 37 °C, and then collected the MNPs by centrifugation. The supernatant was mixed with 2  $\mu$ g plasmid DNA or 20  $\mu$ g BSA and 1% H<sub>2</sub>O<sub>2</sub> and incubated for 1 hour at 37 °C. The MNPs were resuspended in 50  $\mu$ l NaAc (0.1 M, pH 4.5) containing 2  $\mu$ g plasmid DNA or 20  $\mu$ g BSA and 1% H<sub>2</sub>O<sub>2</sub> and incubated for 1 hour at 37 °C. Efficacy of cleavage was then evaluated by electrophoresis.

### Killing of *E. coli* and cleavage of released cellular components

Killing of *E. coli* by the MNP–H<sub>2</sub>O<sub>2</sub> system was tested using TOP10 cells (Invitrogen). TOP10 cells were cultured in liquid LB (with ampicillin, 100  $\mu$ g ml<sup>-1</sup>) medium and incubated at 37 °C overnight; OD600 measured approximately 2.0. Then 20  $\mu$ l of TOP10 cells were spun down and the pellet was resuspended in 50  $\mu$ l NaAc (0.1 M, pH 4.5), containing 20  $\mu$ g of MNPs and 1% H<sub>2</sub>O<sub>2</sub>. After incubation at 37 °C, the mixture was centrifuged, the supernatant was removed, and the pellet was resuspended in 1 ml LB (ampicillin, 100  $\mu$ g ml<sup>-1</sup>) liquid media. OD600 was measured after incubation at 37 °C for 6 hours, reflecting the population size.

To investigate the degradation of DNA and protein released from dead bacteria, *E. coli* cells were boiled at 100 °C. The complex mixture of organic cellular components released by and representing the killed cells was incubated in 50  $\mu$ l NaAc buffer (0.1 M, pH 4.5) containing 20  $\mu$ g MNPs and 1% H<sub>2</sub>O<sub>2</sub>. Nucleic acids were identified by agarose gel electrophoresis and ethidium bromide staining. Protein products were identified *via* SDS-PAGE with Coomassie staining.

### Biofilm elimination

*Pseudomonas aeruginosa* (PA01) was cultured in biofilm minimal media (M63, Amresco) supplemented with 0.2% glucose, 0.5% casamino acids (BD), and 1 mM MgSO<sub>4</sub>, in a 96 well plate overnight at 37 °C. The wells were rinsed with ddH<sub>2</sub>O and dried. Wells were then challenged with minimal media only (positive control), minimal media + MNPs (20  $\mu$ g/50  $\mu$ L), minimal media supplemented with 1% H<sub>2</sub>O<sub>2</sub>, or minimal media supplemented with 1% H<sub>2</sub>O<sub>2</sub> + MNPs. Challenged plates were then incubated for 2 hours at 37 °C with periodic mixing. For the biofilm quantitation assay, the challenged wells were rinsed to remove any remaining planktonic cells and stained with 0.1% crystal violet for 15 minutes. Plates were rinsed to remove the stain and dried. Wells were imaged to visualize biofilm rings that were formed at the air/liquid interface. Subsequently, 10% acetic acid was used to solubilize the dye and then the acetic acid/CV mixture was quantitated using a UV spectrophotometer at 600 nm. The amount of CV present is directly proportional to the number of cells in the biofilm ring.<sup>35</sup> For the viability assay in the biofilm, challenged wells were rinsed to eliminate any remaining planktonic cells. A sterile cotton swab was used to remove cells resident to the biofilm from the well and was subsequently used to inoculate 500  $\mu$ L of M63 salts. Dilutions were made and 100  $\mu$ L aliquots of each dilution were plated onto LB agar plates, and incubated overnight at 37 °C. Colonies were counted and the number of CFUs was compared for each sample.

### Statistics

All experiments were performed in triplicate and data were analyzed using the paired Student's *t*-test. When numerical data are presented as bar graphs, lines denote standard deviations. Additional details on methods can be found in the ESI.†

### Author contributions

L. G., K. M. G., H. S., and A. J. T. conceived of experiments. L. G. and K. M. G. performed the experiments. J. L. N. extracted and purified genomic DNA and RNA. L. G., J. L. N., K. M. G., H. S. and A. J. T. wrote and revised the manuscript.

### Notes

The authors declare no competing financial interest.

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