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# Using biofunctional magnetic nanoparticles to capture Gram-negative bacteria at an ultra-low concentration†

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**After conjugation to vancomycin (Van), chemically stable and highly magnetic anisotropic FePt magnetic nanoparticles ( ~ 4 nm) become water-soluble and capture *E. coli* at 15 cfu mL$^{-1}$.**

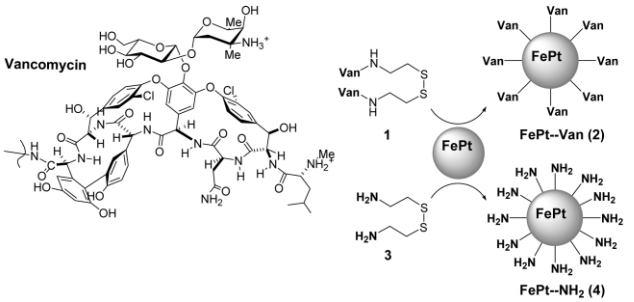
Bacteria at low concentrations (*e.g.*, $< 10^2$ cfu mL$^{-1}$) are hard to detect and usually require long induction times in a culture process to increase their concentrations for detection. To detect bacteria at ultra-low concentrations without time consuming procedures, such as incubation or amplification by polymerase chain reaction (PCR), offers obvious benefits and advantages in clinical diagnosis, environmental monitoring, and food quality control. There is, however, no general and satisfactory assay that could detect bacteria at concentrations less than $10^2$ cfu mL$^{-1}$ without pre-enriching bacteria *via* a culture process.[1] This paper reports a simple process that uses biofunctional magnetic nanoparticles to capture and detect Gram-negative bacteria (the organisms have a cytoplasmic membrane, a cell wall, and an intact outer membrane)[2] within 1 hour at concentration of 15 cfu mL$^{-1}$, which is an order of magnitude more sensitive than one of the best assays for bacteria detection based on luminescence (detection limit: 180 cfu mL$^{-1}$).[3]‡

The field of magnetic nanoparticles has been advancing rapidly in the past few years, partly due to demands for high density magnetic recording materials and breakthroughs in chemical synthesis of magnetic nanoparticles with diameters less than 10 nm.[4,5] Despite the rapid advances in research into magnetic nanoparticles in microelectronics, the applications of magnetic nanoparticles in biomedicine are only just emerging.[6] Magnetic beads (with diameters of 1–5 μm) have been used in biological separations.[7] The smaller size of magnetic nanoparticles, which are 2–3 orders of magnitude smaller than a bacterium, provides extra benefits compared to magnetic beads. When their surface is appropriately elaborated, magnetic nanoparticles can also provide efficient binding to the bacteria because their high surface/volume ratio simply offers more contact area.

To develop a simple and quick assay to detect bacteria at ultra-low concentrations, we have designed a system that combines two kinds of interactions: 1) a magnetic dipole interaction that allows magnetic nanoparticles to aggregate under a magnetic field; and 2) a ligand–receptor interaction that offers tight binding between the magnetic nanoparticles and bacteria when the ligands covalently bond to the surfaces of the magnetic nanoparticles. For example, we attached vancomycin (Van), an antibiotic, to the surface of FePt nanoparticles to capture Gram-positive bacteria (the organisms only have a cytoplasmic membrane and a cell wall)[2] *via* molecular recognition[8] between Van and the terminal peptide, D-Ala-D-Ala, on the surface of Gram-positive bacteria. Although we designed the FePt–Van (**2**) conjugate to bind Gram-positive bacteria,[9] we unexpectedly found that **2** also exhibited selective binding to Gram-negative bacteria at a very low concentration, which will be described in this work.

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/cc/b3/b305421g/

Scheme 1 illustrates the synthetic route for making the FePt–Van nanoparticles: bis(vancomycin) cystamide[10] (in aqueous solution) reacts with FePt nanoparticles[5] (in hexane phase) under vigorous stirring for 12 hours to form Pt–S and Fe–S bonds that link Van to FePt. After the reaction, the product, **2**, becomes water soluble, and can be easily separated from the organic phase. FePt nanoparticles also react with cystamine to give FePt–$NH_2$ (**4**) as a control compound. Detailed synthesis and characterization of **2** and **4** will be reported separately.




**Scheme 1** The synthesis of vancomycin conjugated FePt nanoparticles (**2**) and the control (**4**).

Fig. 1 shows the general experimental procedure. After adding the aqueous solution of **2** (13 μg mL$^{-1}$) or **4** (15 μg mL$^{-1}$) into a vial of solution containing *E. coli* (15 cfu mL$^{-1}$) and shaking for 20 minutes, we applied a magnetic field ( ~ 3000 G) to the solution. Upon the application of the magnetic field, the magnetic nanoparticles aggregated irreversibly due to the high magnetic anisotropy of the FePt nanoparticles. Despite this being sometimes an annoying phenomenon in the synthesis of magnetic nanoparticles (*e.g.*, FePt or $SmCo_5$),[5,11] it turned out to be very useful for increasing the load of magnetic nanoparticles attached to the bacteria (*vide infra*), thus providing an adequate force to "focus" the bacteria into a small area when a small magnet is used. Then the remaining solution was removed and the aggregates were washed thoroughly using deionized water and transferred to a glass slide for microscopic study.

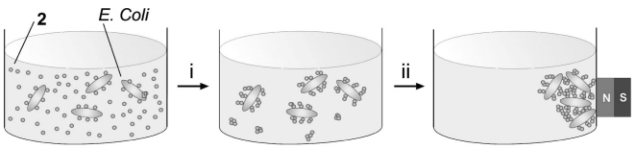



**Fig. 1** Illustration of the capture of *E. coli* by **2**. (i) The application of magnetic field induced aggregation and (ii) the attraction of "magnetized" *E. coli* by a small magnet.

Fig. 2A shows the optical image that indicates the aggregates of *E. coli* and **2** when **2** is used as the capture agent. In contrast, optical microscopy suggests that there was no *E. coli* captured by the magnetic nanoparticles when **4** is used (Fig. 2B). This observation suggests that **2** binds to *E. coli* selectively. Though the exact binding mechanism is unclear at this moment, we speculate that the glycoside moieties of Van on **2** may bind to

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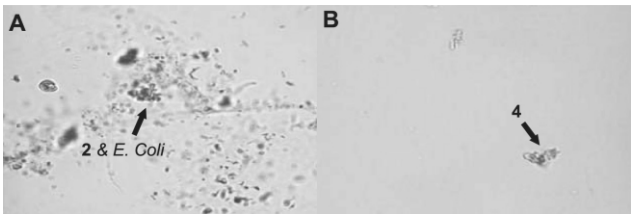


**Fig. 2** Optical images (magnification ×400) of (A) the aggregates of *E. coli* and **2** and (B) the aggregate of **4**.

some unknown receptors on the outer membrane of *E. coli*. Such binding seems to be specific enough to distinguish *E. coli* from white blood cells (WBC) since we found that neither **2** or **4** captures WBC.

To further confirm the result obtained by an optical microscope, we used scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to study the aggregates. In Fig. 3A, SEM shows that the *E. coli* indeed aggregates with the nanoparticles **2**, and it is easy to distinguish them because the shape and morphology of *E. coli* differ dramatically from those of **2**. In the case of **4**, only the aggregates of **4** are observed by using SEM (Fig. 3B). The absence of *E. coli* further confirms the selectivity of **2** towards *E. coli*. In Fig. 3C, TEM reveals features of the binding between

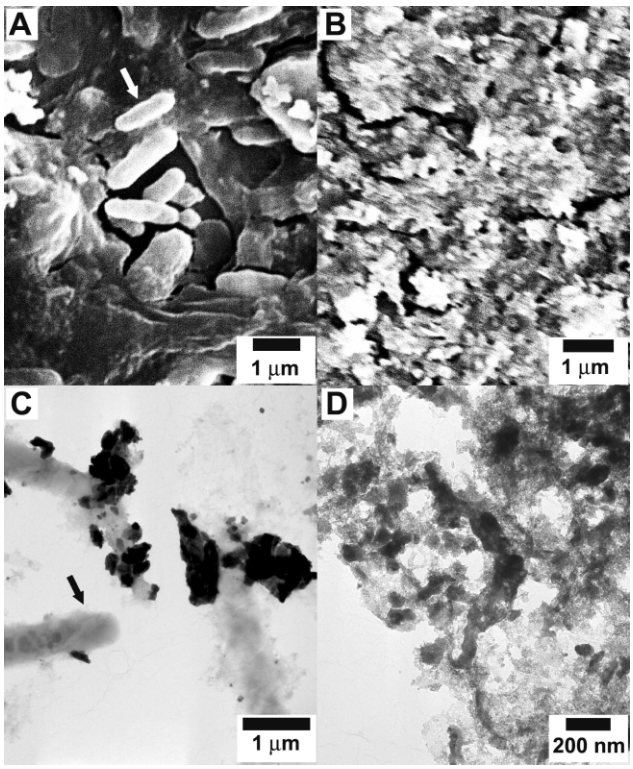


**Fig. 3** The SEM images of (A) aggregates of *E. coli* and **2**, and (B) aggregates of **4** nanoparticles; the TEM images of (C) aggregates of *E. coli* and **2**, and (D) aggregates of **4** nanoparticles (arrows indicate the *E. coli*).

**2** and *E. coli*: Instead of uniformly covering the *E. coli*, the aggregates of **2** attach to the bacterium in several locations. This observation indicates that **2** initially binds to the *E. coli* cells sporadically because of the low concentration of the bacteria; in the process of forming magnetic aggregates due to magnetic dipolar interaction, the magnetic field induces more magnetic nanoparticles to attach to those already on the bacteria. With enough magnetic nanoparticles on them, the *E. coli* cells are attracted easily by the small magnet. Fig. 3D, again, confirms that **4** forms aggregates but fails to bind to the *E. coli* cells.

In conclusion, by combining FePt magnetic nanoparticles with vancomycin, we have demonstrated a sensitive and quick assay to allow bacteria to be captured and analyzed, though the exact mechanism by which **2** binds to Gram-negative bacteria, such as *E. coli*, has yet to be elucidated. The existing archives of optical and SEM profiles of most bacteria allow easy identification of captured bacteria. The high sensitivity provided by magnetic nanoparticles will allow detection of other biological substrates at exceedingly low concentrations.

## Notes and references

‡ The exact counts of the *E. coli* were confirmed by back titration.

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