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Supporting Information

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µHall Chip for Sensitive Detection of Bacteria

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Supplementary Methods

Fabrication of µHall system

The details regarding the design and fabrication of the device are similar to those described previously[1], with the exception that the present system was adapted to detect much smaller targets (bacteria). Briefly, the device consists of microfabricated Hall sensors built upon a gallium arsenide (GaAs) substrate with a polydimethylsiloxane (PDMS) microfluidic network built directly on top. The substrate contains an epitaxially grown pseudomorphic high electron mobility transistor (PHEMT) heterostructure (IntelliEpi). Photolithography was used to define the mesa, followed by an anisotropic reactive ion etch. Electrodes were patterned with photolithography, and metal layers were deposited using thermal evaporation: Ni (50 Å), Au (50 Å), Ge (250 Å), Au (400 Å), Ni (100 Å), Au (400 Å). The electrodes were annealed at 480 ℃ for 90 seconds in a rapid thermal annealer to form a eutectic alloy. Passivation layers were deposited in the following order to protect the sensor from biological media: 1) 30 nm Al_2O_3, grown by atomic layer deposition (ALD), 2) 100 nm Si_3N_4 grown by chemical vapor deposition (CVD), and 3) 100 nm SiO_2 grown by CVD to form a layer capable of forming permanent bonds with PDMS. The microfluidic network was fabricated using soft lithography. Two-step photolithography was used to fabricate a two-layer SU-8 (MicroChem) mold. PDMS was poured onto the mold and cured at 65 ℃ for 3 hours. The PDMS microfluidic network and GaAs chip were activated using O_2 plasma, aligned using a modified mask aligner, and permanently bonded.

Magnetic simulation for µHall sensing

A numerical model was constructed to describe the spatial response of the µHall sensors to the magnetic moments of passing bacteria. A magnetically labeled bacterium was approximated as a dipole moment located at the centroid of the cell. The magnetic field

normal to the sensor's surface $B_\perp$, produced by the magnetic dipole at a specific position (x, y, z), was calculated analytically. The Hall voltage was then obtained for a given bias current I, by integrating $B_\perp$ over the area of the Hall sensor. This numerical model was used to determine the change in V_H measured by the Hall sensor as the dipole was moved away from the sensor surface. The V_H signal was thus observed to drop off as the distance d increased (**Fig. 2c**), showing an r^{-3} dependency. For $d < 2$ μm, the signal leveled off as most of the magnetic flux from the dipole was captured by the Hall sensor. Simulations were performed in MATLAB (Mathworks).

Preparation of labeling agents (vanc-TCO and MNP-Tz)

Trans-cyclooctene-modified vancomycin (vanc-TCO) was synthesized as described previously.[2] In brief, 8.4 mg of vancomycin (Sigma-Aldrich) in dimethylformamide (DMF) was added to a solution of *trans*-cyclooctene N-hydroxy-succinimidyl ester (TCO-NHS, 4 mg) and trethylamine (58 μmol) in DMF, and reacted for 6 hours. The product was then analyzed by high performance liquid chromatography (HPLC; Waters). Magnetic nanoparticles (MNPs) were synthesized according to a previous report.[3] The MNPs consisted of an iron oxide ($(Fe_2O_3)_m(Fe_3O_4)_n$) core and a shell of crosslinked dextran, which produced a hydrodynamic diameter of 21 nm. Each particle had 8 fluorescein molecules and 22 free amine groups on its surface. Tetrazine-*N*-hydroxysuccinimide (Tz-NHS) was synthesized as described previously.[4] For preparation of Tz-conjugated MNPs (MNP-Tz), Tz-NHS dissolved in 1 volume of DMSO was added, in 500 molar excess to MNPs, in 9 volume of phosphate buffered saline (PBS) containing 10 mM sodium bicarbonate, and reacted at room temperature for 4 hours. Unreacted Tz-NHS was removed using the Amicon centrifugal filters (GE Healthcare).

Supplementary References

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Supplementary Figure

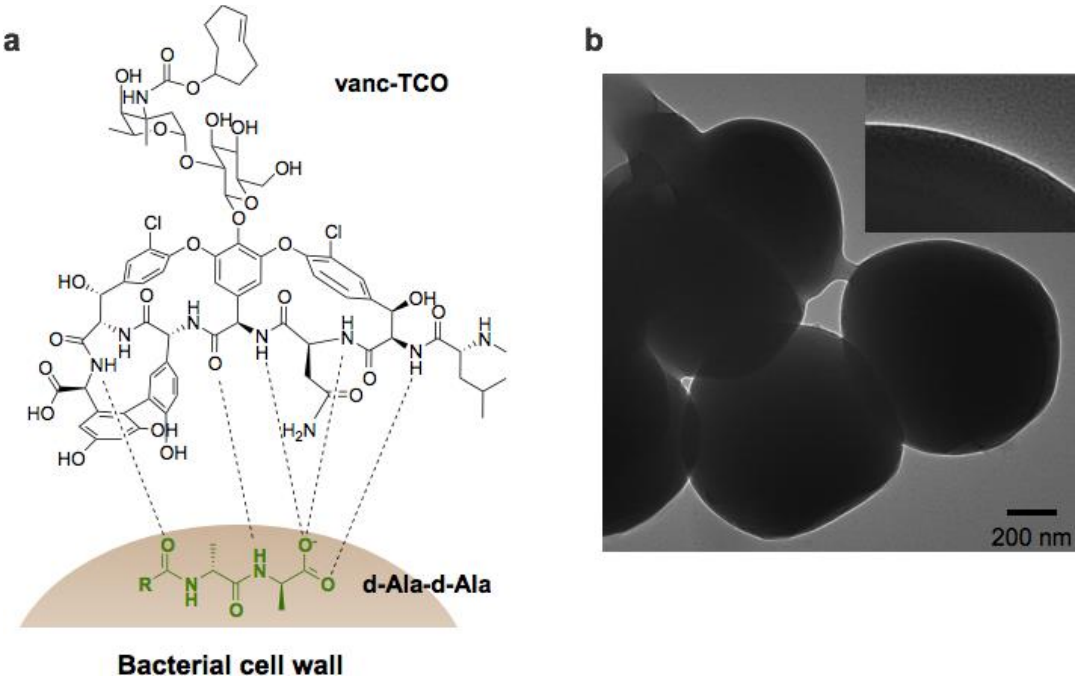

Figure S1. Bacterial targeting using *trans*-cyclooctene-modified vancomycin (vanc-TCO). (a) Vancomycin, which binds to d-Ala-d-Ala moieties in Gram-positive bacterial cell wall, was used as the labeling agent. The antibiotic was derivatized with *trans*-cyclooctene (TCO) to provide a facile handle for coupling with tetrazine (Tz)-modified MNPs. (b) Control bacterial samples (*S. aureus*) were prepared without pre-targeting with vanc-TCO, and showed negligible particle binding following incubation with MNP-Tz.