Supporting Information

Chimeric phage-nanoparticles for rapid characterization of bacterial pathogens: detection in complex biological samples and determination of antibiotic sensitivity

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Text S1.

The receptor-binding protein of CTXϕ binds to the toxin-coregulated pilus (TCP). In general, the epidemic-causing *V. cholerae* serogroups O1 and O139 generally express TCP, while non-O1, non-O139 serogroups do not.[1] Thus the modified phage M13-g3p(CTXϕ) is expected to recognize TCP-expressing cells, primarily O1 and O139 serotypes.

The receptor-binding protein of Pf1 binds to the type IV PAK pilus, expressed by *P. aeruginosa* strain K (PAK). The PAK strain is a hyperpiliated, virulent, well-characterized laboratory strain.[2] Thus the modified phage M13-g3p(Pf1) is expected to recognize PAK strains but not necessarily others (e.g., PAO strains).

The receptor-binding protein of M13 is the conjugative F pilus of *E. coli*.[3] M13 therefore recognizes *E. coli* F+ strains but not F- strains.

Figure S1. Characterization of AuNPs. Representative TEM images of AuNPs with size of (a) 7 nm, (b) 20 nm, (c) 50 nm and (d) 85 nm. Scale bar = 50 nm. (e) Size distribution measured by DLS (intensity-weighted).

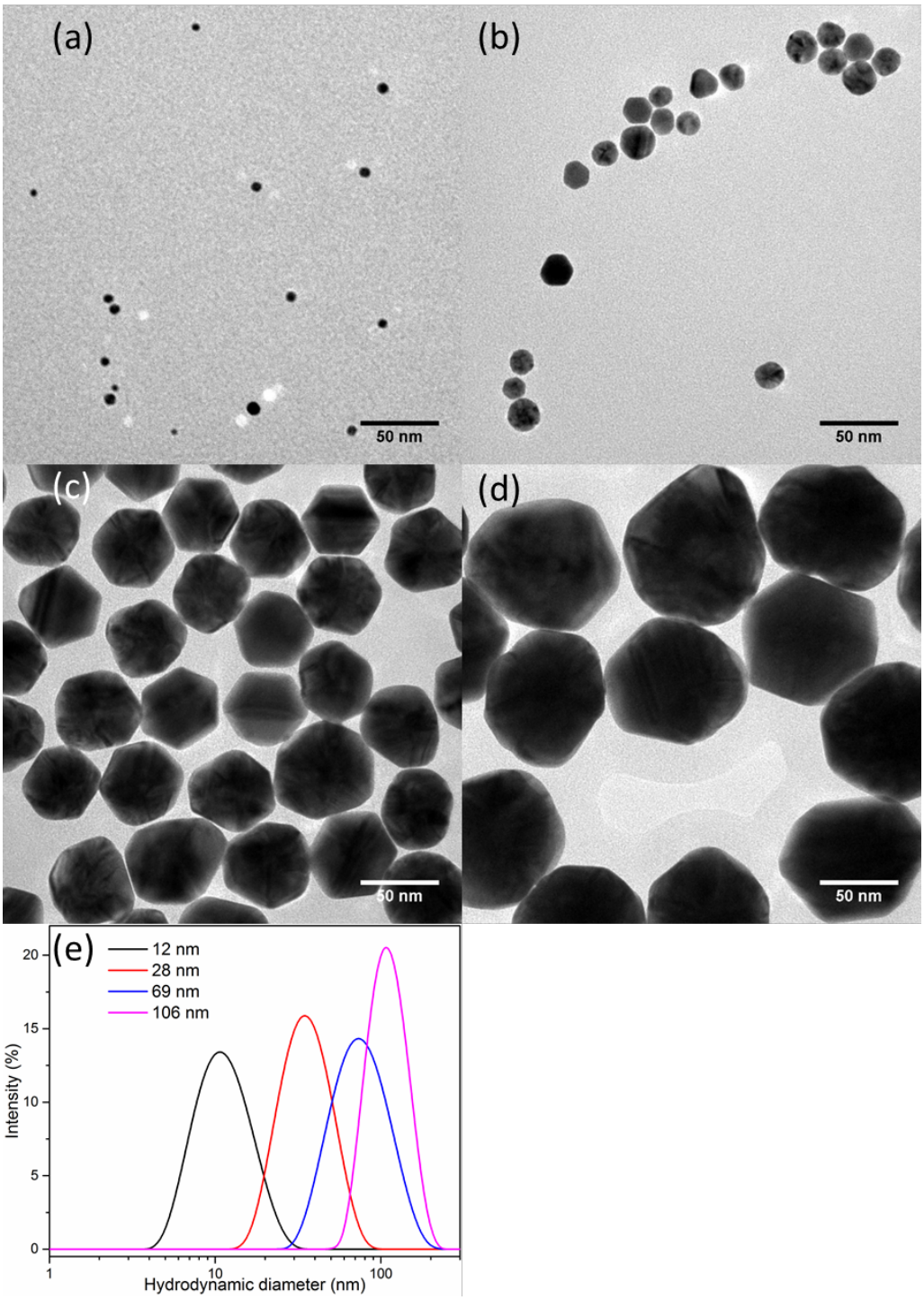


Figure S2. Characterization of thiolated phage. ATR-FTIR of M13KE phage before (blue) and after (red) thiolation shows increased signals of S–H stretching (2550 cm^{-1}) and C–S stretching (659 cm^{-1}) after thiolation. The ζ of the phage decreased from −44.3 mV to −9.52 mV after modification, also confirming successful chemical modification of the phages.

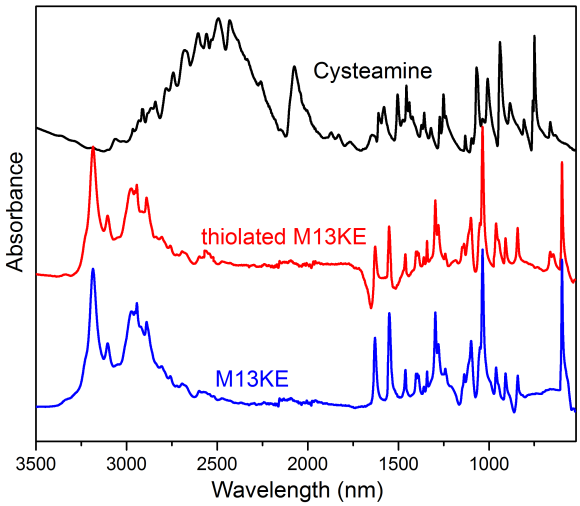


Figure S3. Limit of detection of *P. aeruginosa* in drinking water with thiolated M13-g3p(Pf1). (a) Digital photos and (b) UV–vis spectra are shown. Samples from left to right in (a) are: AuNPs with no bacteria or phages, ~40 CFU *P. aeruginosa* with unmodified M13-g3p(Pf1) and AuNPs (control), ~40 or ~10 CFU of *P. aeruginosa* with thiolated M13-g3p(Pf1) and AuNPs. Note the amount of cells is estimated based on the dilution series, so the number of cells in a ~10 or ~40 CFU sample were estimated by calculation and should be taken as approximations.

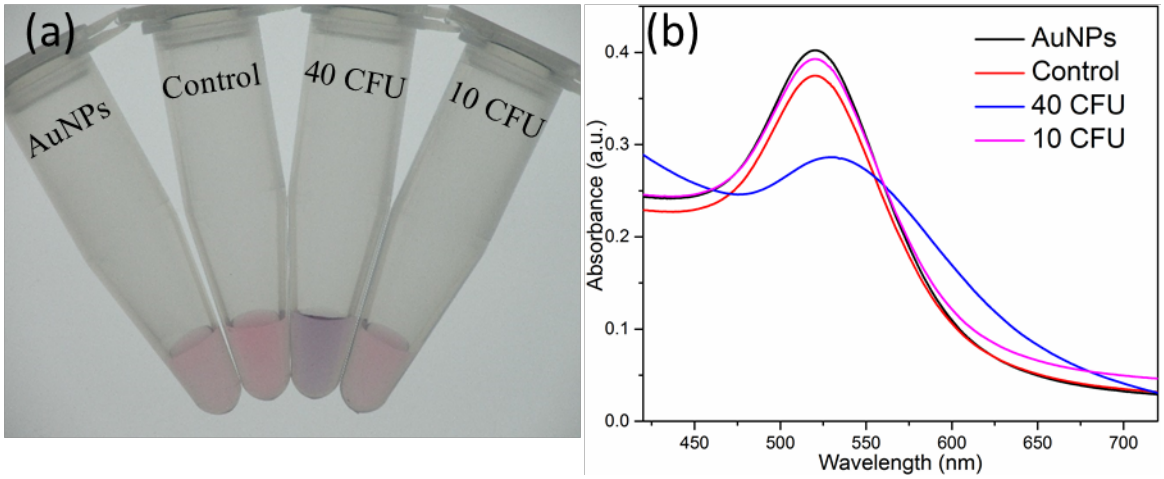


Figure S4. Detection of *V. cholerae* with thiolated M13-g3p(CTXϕ) and AuNPs in (a, d) drinking water, (b, e) fat-free milk, and (c, f) urine. (a, b, c) Digital photos and (d, e, f) UV–vis spectra are shown. Samples from left to right in each photo are: AuNPs with no bacteria or phages, unmodified M13-g3p(CTXϕ) phage with 10^6 CFU *V. cholerae* (control), and thiolated M13-g3p(CTXϕ) phage with *V. cholerae* at 10^2, 10^4, and 10^6 CFU, respectively.

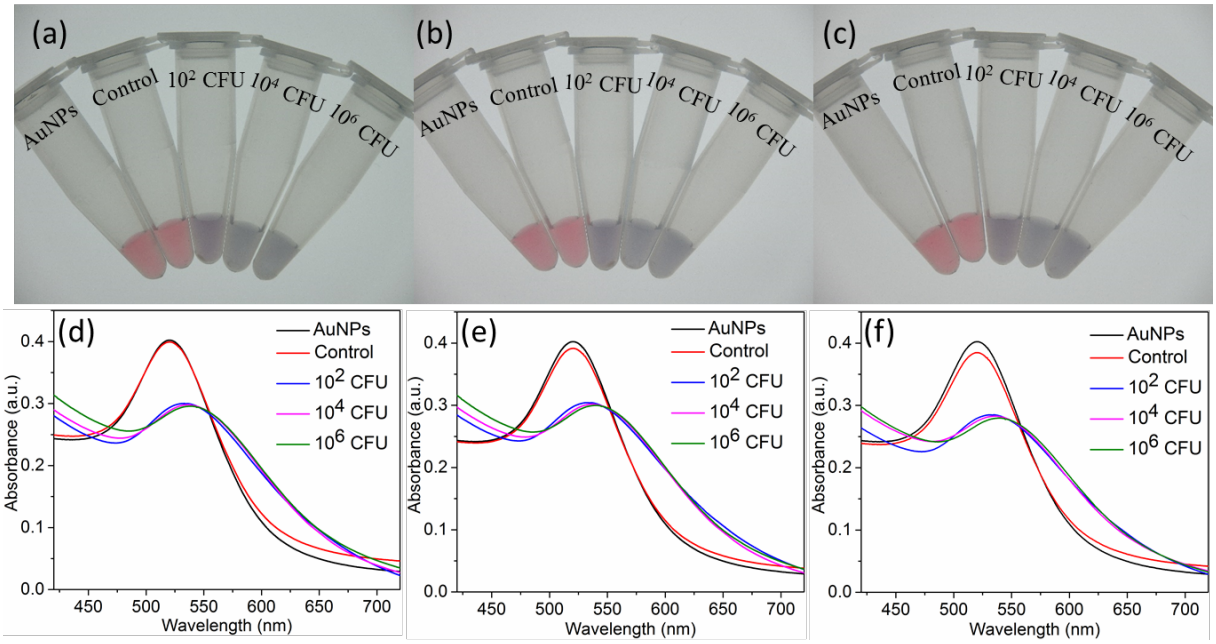


Figure S5. Detection of *E. coli* ER2738 with thiolated M13KE and AuNPs in (a, d) drinking water, (b, e) fat-free milk, and (c, f) urine. (a, b, c) Digital photos and (d, e, f) UV–vis spectra are shown. Samples from left to right in each photo are: AuNPs with no bacteria or phages, unmodified M13KE phage with 10^6 CFU *E. coli* ER2738 (control), and thiolated M13KE phage with *E. coli* ER2738 at 10^2, 10^4, and 10^6 CFU, respectively.

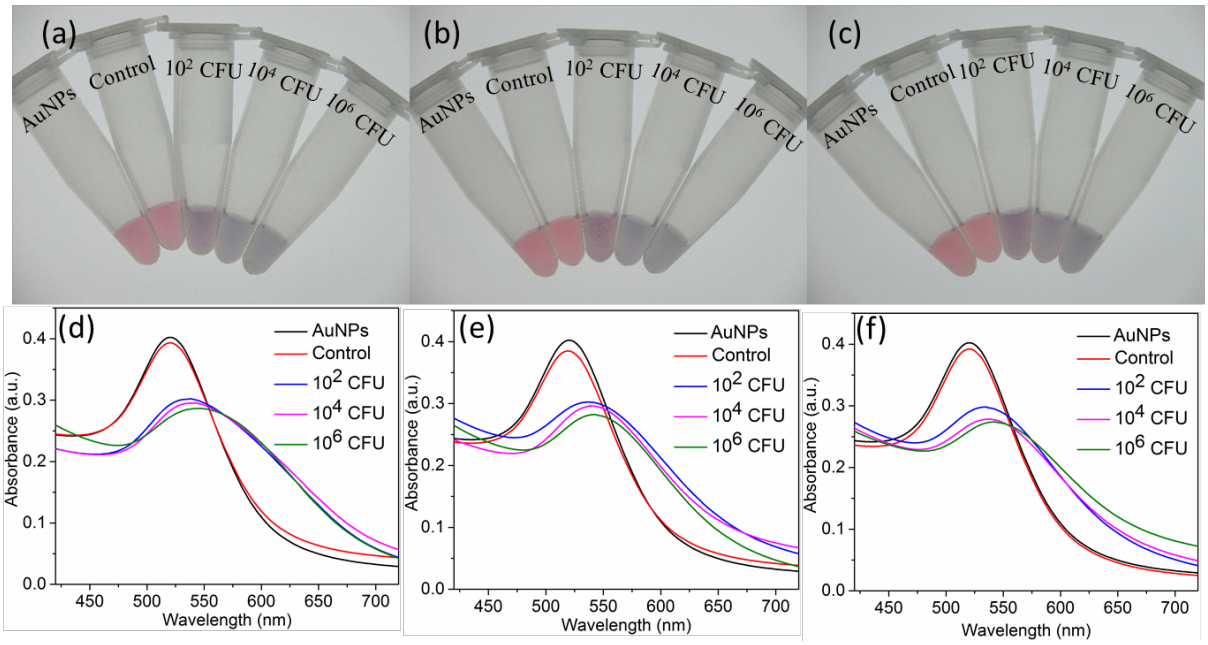


Figure S6. Relationship between SPR absorbance and bacterial concentration (*E. coli* ER2738 in tap water). The spectra were analyzed in two ways: (a) by determining the wavelength of the peak maximum, which shifts from 520 nm (non-aggregated AuNPs) to ≥ 530 nm (aggregated AuNPs); and (b) the ratio of the absorbance at the SPR peak compared to the absorbance at 520 nm.

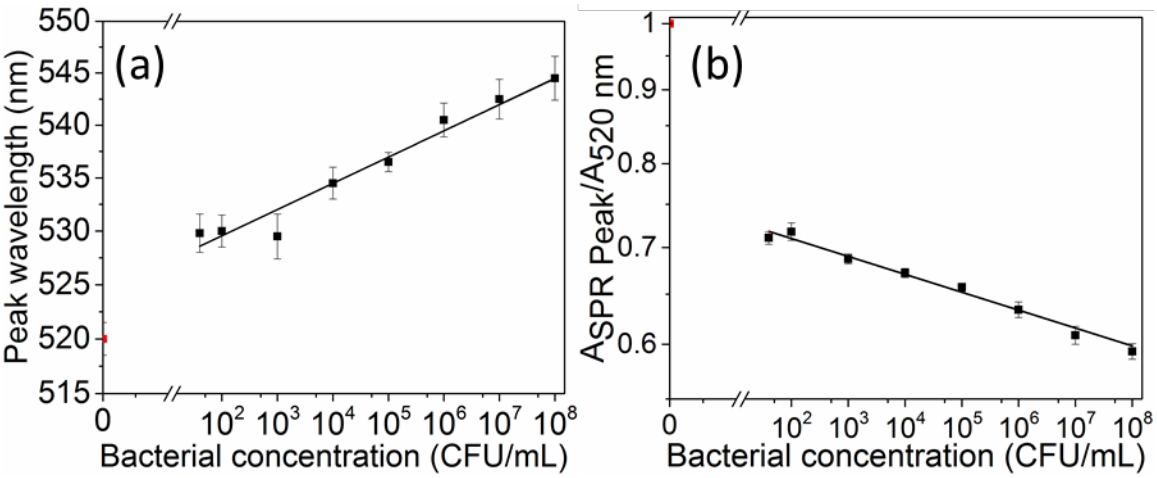


Figure S7. TEM images of (a) *P. aeruginosa* and (b) non-thiolated M13-g3p(Pf1) phage exposed to AuNPs, demonstrating no aggregation of AuNPs on either bacteria or phage.

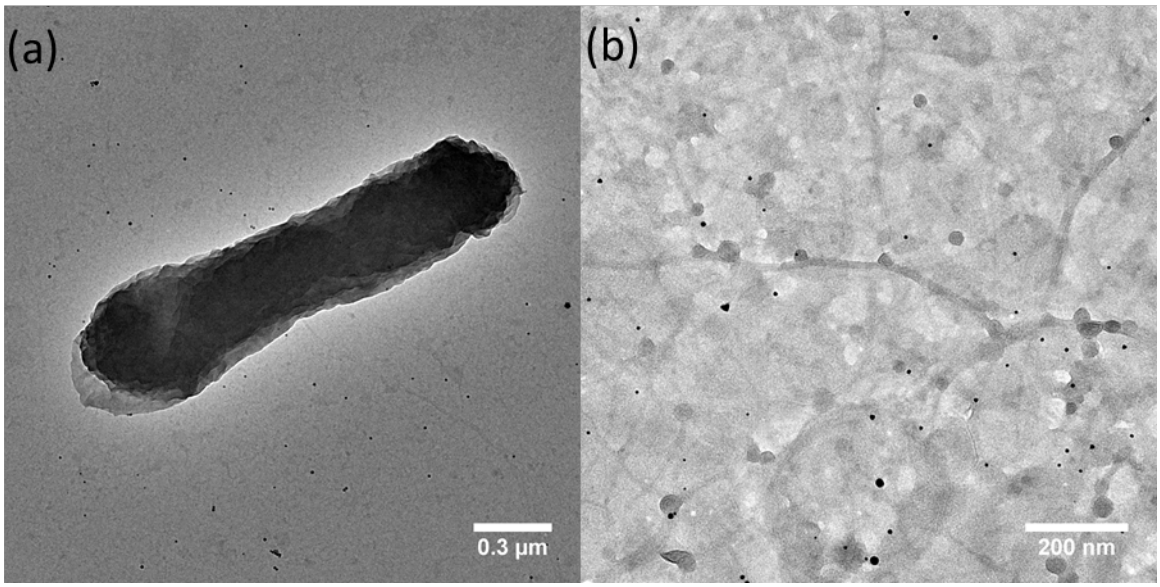


Figure S8. Lack of cross-reactivity for different bacterial species. The specificity of bacterial detection of each phage was verified by conducting the assay in the presence of non-host bacteria. Absorption spectra of (a) M13-g3p(Pf1), (b) M13-g3p(CTXϕ), (c) M13KE when incubated with different bacterial species (10^6 CFU) and AuNPs. Bacterial species shown are *P. aeruginosa* (red), *V. cholerae* 0395 (green), and *E. coli* ER2738 (blue). The spectrum of AuNPs alone (dashed black line) is also shown.

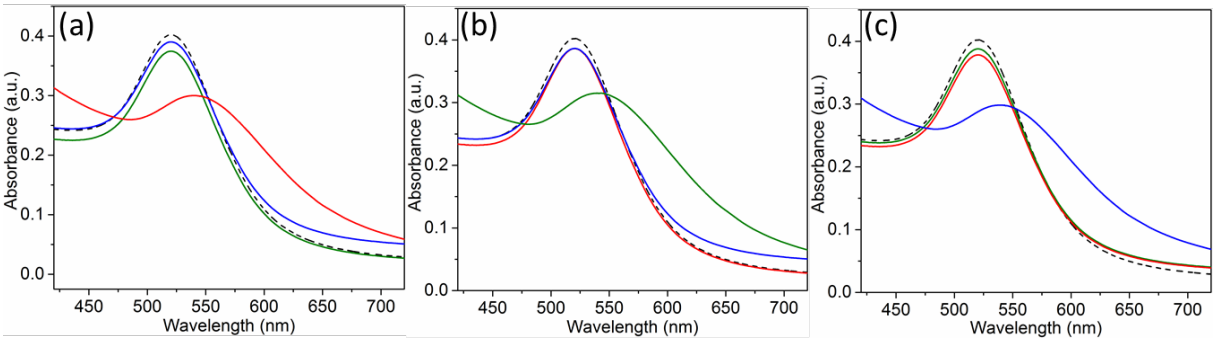


Figure S9. Specificity of bacterial detection in the context of a heterogeneous mixture of bacteria (*P. aeruginosa*, *V. cholerae*, and *E. coli* ER2738). Digital photos (top row) and UV–vis spectra (bottom row) are shown. Phages tested were (a, d) M13-g3p(Pf1), (b, e) M13-g3p(CTXϕ), and (c, f) M13KE. Samples from left to right in each photo are: AuNPs with no bacteria or phages, unmodified phage in the mixture of bacteria containing 10^6 CFU target cells and 10^6 CFU of non-target cells (control), and thiolated phage in the mixture of bacteria containing 10^6 CFU of non-target cells and target cells of 10^2, 10^4, and 10^6 CFU, respectively.

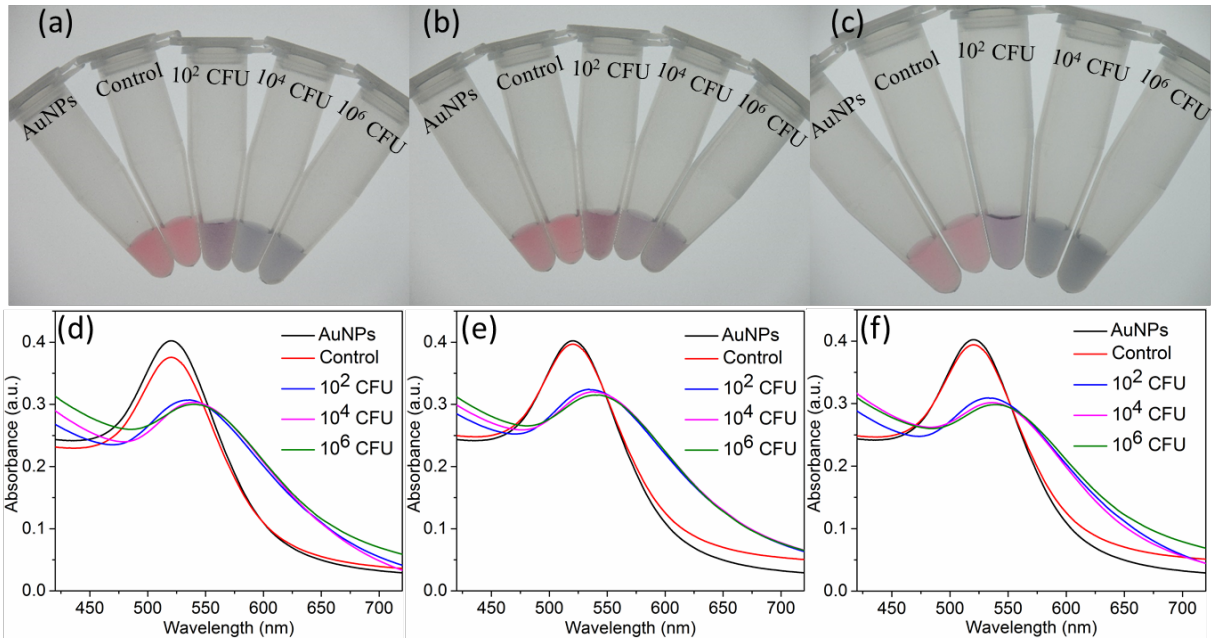


Figure S10. Detection of *P. aeruginosa* with thiolated M13-g3p(Pf1) and AuNPs in **non-filtered** fat-free milk. (a) Digital photos and (b) UV–vis spectra are shown. Samples from left to right in the photo are: AuNPs with no bacteria or phages, unmodified M13-g3p(Pf1) phage with 10^6 CFU *P. aeruginosa* (control), and thiolated M13-g3p(Pf1) phage with *P. aeruginosa* at 10^2, 10^4, and 10^6 CFU, respectively. Similar results were obtained with cells added to **filtered** fat-free milk (Figure 2b,e).

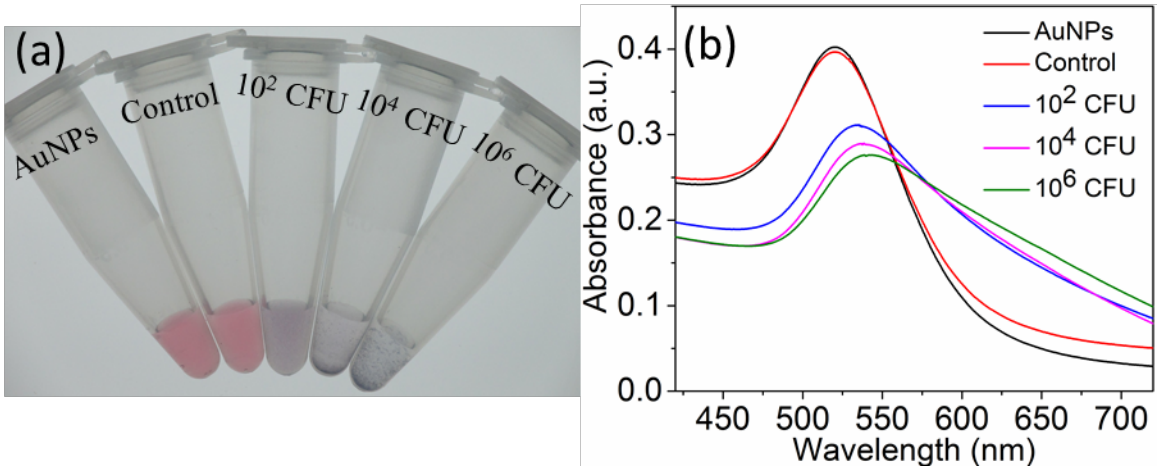


Figure S11. Standard calibration curve of concentration of *E. coli* ER2738 vs absorbance at 450 nm determined by QuickDetect^{TM} E. coli Protein (ECP) ELISA Kit (BioVision, CA, USA). Fitting equation: $y = x$ *6.63 x 10^{-6}.

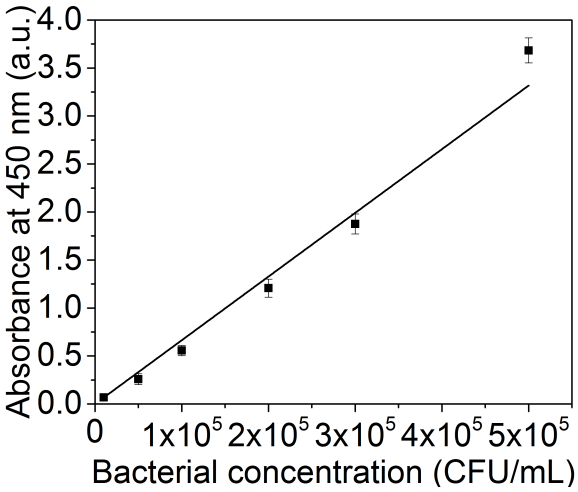


Figure S12. Lack of detection of *P. aeruginosa* with larger AuNPs and thiolated M13-g3p(Pf1) in tap drinking water. (a, b, c) Digital photos and (d, e, f) UV–vis spectra are shown. Samples from left to right in each photo are: AuNPs with no bacteria or phages, 10^6 CFU *P. aeruginosa* with unmodified M13-g3p(Pf1) and AuNPs (control), and 10^6 CFU of *P. aeruginosa* with thiolated M13-g3p(Pf1) and AuNPs. The sizes of AuNPs used were (a, d) 20 nm, (b, e) 50 nm, and (c, f) 85 nm, respectively.

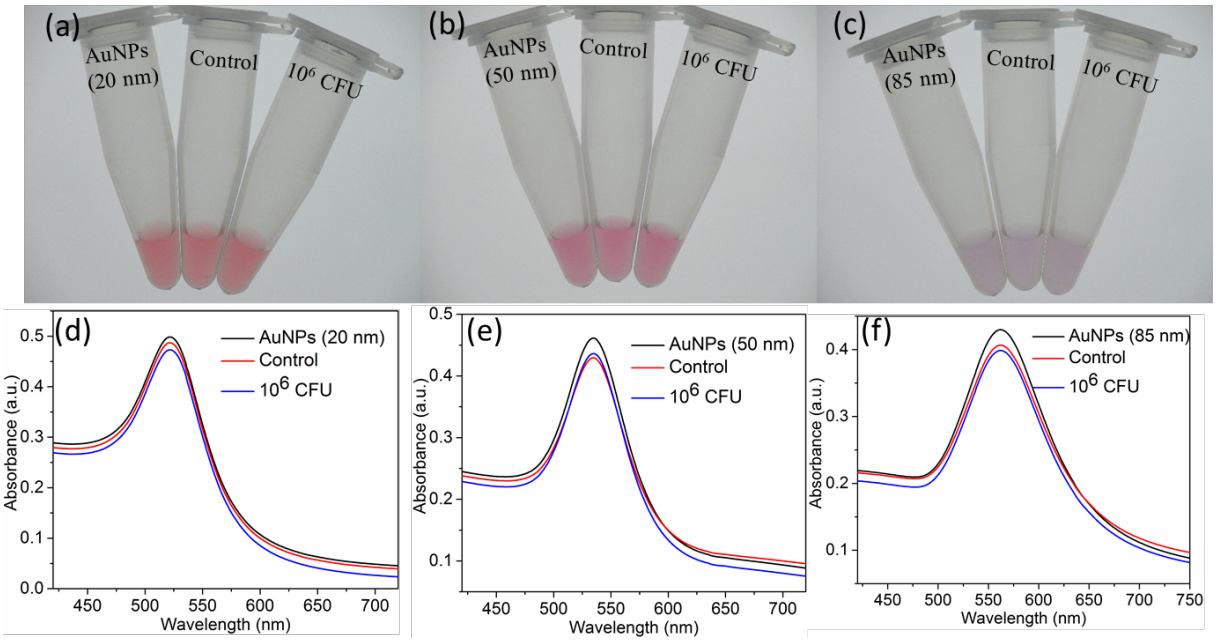


Figure S13. Lack of detection of *P. aeruginosa* in tap drinking water with M13-g3p(Pf1) and HOOC-PEG-modified AuNPs (7 nm). (a) Digital photo and (b) UV–vis spectra are shown. Samples from left to right in (a) are: PEG-AuNPs with no bacteria or phages, 10^6 CFU *P. aeruginosa* with unmodified M13-g3p(Pf1) and PEG-AuNPs (control), 10^6 CFU of *P. aeruginosa* with thiolated M13-g3p(Pf1) and PEG-AuNPs.

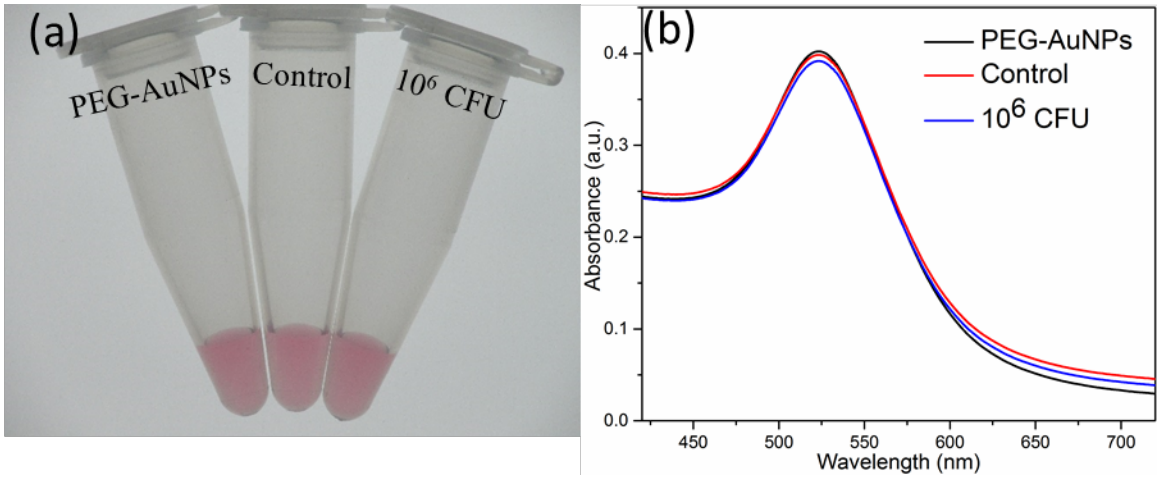
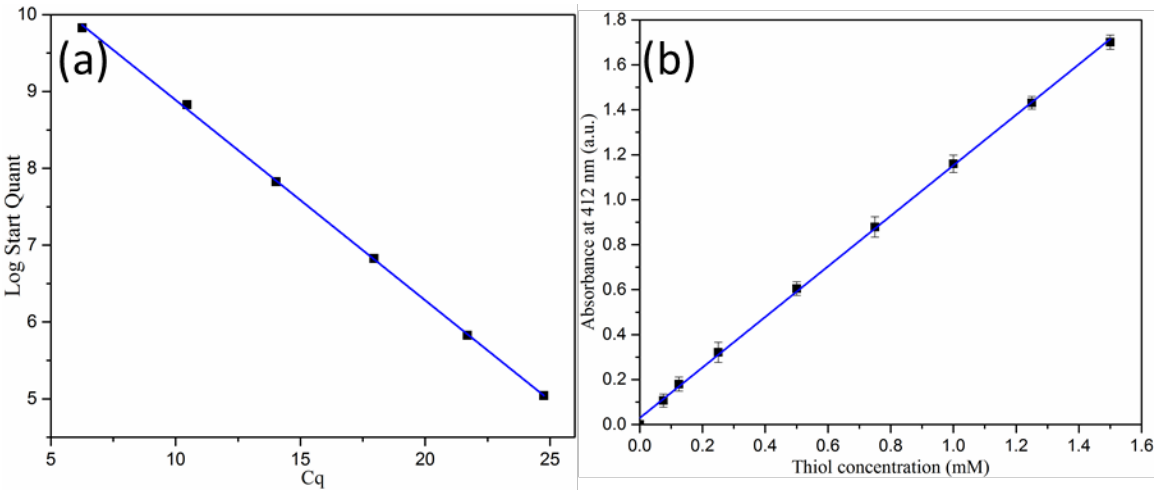


Table S1. Number of thiol groups per chimeric phage after chemical modification, quantified by Ellman's assay and real-time PCR (Figure S14).

Phage	No. of thiol groups per phage
M13-g3p(Pf1)	2809 ±198
M13-g3p(CTXϕ)	2257±207
M13KE	2358±256

Figure S14. Standard curves of (a) real-time PCR of M13 phages, and (b) Ellman's assay for thiol group quantification using absorbance measurement at 412 nm.



Supporting Methods

Phage propagation. To propagate M13KE, a single colony of *E. coli* ER2738 was cultured in LB broth with 10 μg/mL tetracycline in a shaking incubator at 37 °C overnight. 200 μL of the overnight *E. coli* culture was inoculated in 20 mL LB in a 250 mL Erlenmyer flask. 1 μL M13KE phage stock solution (10^{13} pfu/mL) was added to the cell culture and propagated at 37°C at 250 rpm for 3 h. The 20 mL cell culture was split into three aliquots and each of them was inoculated in 330 mL LB in a 2 L flask. The flasks were then shaken at 37°C, 250 rpm for another 4 -5 h. The cells were spun down at 5000 rpm for 15 min, and the supernatant was centrifuged again (8000 rpm, 15 min) to remove cell debris. The supernatant was collected and wild type M13KE phage was precipitated and purified by PEG/NaCl precipitation [4].

Propagation of bacteria. To propagate *E. coli* ER2738, *Vibrio cholerae* [5] and *P. aeruginosa* (Schroeter) Migula (ATCC 25102), a single colony was inoculated into 5 mL LB medium, either with tetracycline (10 μg mL^{-1}, for ER2738) or without antibiotics, in a 50 mL Falcon tube and shaken at 37 °C overnight for 24 h. The bacterial cell density was determined by measuring the optical density at 600 nm (OD_{600}). Corresponding cell densities were estimated as described previously [4].

Supporting References

1. Goel, A. K.; Jiang, S. C., Genetic determinants of virulence, antibiogram and altered biotype among the Vibrio cholerae O1 isolates from different cholera outbreaks in India. *Infect Genet Evol* **2010,** *10* (6), 815-819.
2. Cain, A. K.; Nolan, L. M.; Sullivan, G. J.; Whitchurch, C. B.; Filloux, A.; Parkhill, J., Complete Genome Sequence of Pseudomonas aeruginosa Reference Strain PAK. *Microbiol Resour Ann* **2019,** *8* (41).
3. Deng, L. W.; Perham, R. N., Delineating the site of interaction on the pIII protein of filamentous bacteriophage fd with the F-pilus of Escherichia coli. *J Mol Biol* **2002,** *319* (3), 603-614.
4. Peng, H.; Chen, I. A., Rapid Colorimetric Detection of Bacterial Species through the Capture of Gold Nanoparticles by Chimeric Phages. *Acs Nano* **2019,** *13* (2), 1244-1252.
5. Martinez, R. M.; Megli, C. J.; Taylor, R. K., Growth and laboratory maintenance of Vibrio cholerae. *Curr Prot Microb* **2010,** *Chapter 6*, Unit-6A.1.