Supporting Information for

Chemically individual armoured bioreporter bacteria used for *in vivo* sensing of ultra-trace toxic metal ions

Experimental section

Bacteria culture: *Escherichia coli* DH5α (DH5α) were cultured in Luria-Bertani (LB) culture medium at 37 ℃ under shaking. *Escherichia coli* DH5α bioreporter strain 1598 was cultured in LB medium with 50 μg mL^{-1} at 37 ℃ under shaking. The egfp expressed 1598 (1598^{egfp}) were obtained through sodium arsenite induction. Briefly, 1598 were first cultured in LB (with Kanamycin) at 37 ℃ until to a bacterial optical density about 0.5 at 600 nm, and then sodium arsenite was added with a final concentration of 1 mg L^{-1}. After that, the suspension was shook at 30 ℃ for another 8 h for egfp expression and harvested by centrifugation at 3000 rpm for 10 min. Notably, due to the weak background fluorescence of 1598, in the present work, the *E coli* strain DH5α was used for the vaibility and permeability test. Green fluorescent bacteria 1598^{egfp} and 1598^{egfp}@mSi were used in the phagocytosis assay.

Bacteria encapsulation: The bacteria (DH5α, 1598 or 1598^{egfp}) were encased with mesoporous silica through a one-pot synthesis method. Typically, bacteria were separated from the culture medium by centrifugation at 3000 rpm for 10 min. The bacteria pellet was washed with 10.3% saccharose isotonic solution twice and resuspended in 10.3% saccharose. Then the suspension was poured into the isotonic solution containing 2 mg mL^{-1} Pluronic®F-127, 3 μmol (3-aminopropyl) triethoxysilane (APTES) and 56 μmol tetraethoxysilane (TEOS) with a final bacterial optical density of 0.25 at 600 nm. After agitating at room temperature over night, the white precipitates were isolated at 3000 rpm for 2 min, and after washisng the final product were resuspended in LB medium and storaged at 4 ℃ for further use.

Bacteria that encased with silica shell through conventional encapsulation method were also prepared in pure water without Pluronic®F-127.

Bacterial viability test: The bacterial viability was measured by fluorescein diacetate (FDA) - propidium iodide (PI) double staining method. Typically, 2 μL of the FDA stock solution (10 mg mL^{-1} in acetone) and 1 μL of PI stock solution (1 mg mL^{-1} in deionized water) were added to 1 mL pH 7.4 PBS buffer containing bacteria with an optical density of 0.2 at 600 nm. The suspension was incubated at 37 ℃ for 30 min, and then separated by centrifugation. After washing with the PBS buffer the bacteria were characterized by fluorescence microscope. Fluorescence imaging of bacteria were performed with an Olympus BX-51 optical system microscope (Tokyo, 30 Japan).

Evaluation of bacterial proliferation: 1598, 1598@mSi and 1598@Si with the initial concentration of 10^5 CFU mL^{-1} (10 μL suspension with the optical density of 1.0 at 600 nm was diluted to 10 mL) was cultured in LB culture medium (50 μg mL^{-1} Kanamycin) at 37 ℃ under shaking. The bacterial concentrations were determined at intervals.

Anti-insults test: The two representative insults lysozyme (Lys) and silver nanoparticles (AgNPs) (15-30 nm) were used to test the anti-insults capability of the bacteria. Typically, 1 mL pH 7.4 PBS buffer containing bacteria (DH5α, DH5α@mSi) with an optical density of 0.2 at 600 nm was put into a 1.5 mL PE tube. Then Lys or AgNPs was added into the tube with a final concentration of 50 μg mL^{-1} (Lys) and 20 μg mL^{-1} (AgNPs), respectively. After incubation at 37 ℃ for one hour the bacteria

were separated by centrifugation at 3000 rpm and washed with buffer. Then the bacteria were resepended for fluorescence imaging.

Phagocytosis assay: Murine macrophage cell line RAW264.7 was used as the model of phagocyte. Cells were cultured in Dul-becco's modified Eagle's (DMEM) (Gibco BRL) medium supplemented with 10% fetal bovine serum (FBS) in a 5% CO_2 humidified environment at 37 ℃ for an appropriate time. For the flow cytometry, RAW264.7 cells were seeded at a density of 5×10^5 cells/well in 6-well assay plates and allowed to 4 h grow for attachment. Then different concentrations of 1598^{egfp} or 1598^{egfp}@mSi were added with final bacteria to RAW264.7 ratio of approximately 10:1 and 100:1 respectively. After 2 h incubation and three times washing, the cells were harvested by trypsin digestion and resuspended in 1 mL PBS for flow cytometry test. Flow cytometry was recorded on a BD FACSCalibur flow cytometer (BD Biosciences, U.S.A.). For confocal imaging, RAW264.7 cells were seeded at a density of 1×10^5 cells/well in 24-well assay plates with a cover glass each in the bottom and allowed to 4h grow for attachment. Then 1598^{egfp} or 1598^{egfp}@mSi was added with final bacteria to RAW264.7 ratio of approximately 100:1. After 2 h incubation, the washed cells were stained with Hoechst for 30 min and then 10 μg mL^{-L} 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) for 10 min at room temperature before washing with PBS for three times. Then the fluorescence images were observed by laser scanning confocal microscopy (CLSM; LSM 700, Carl Zeiss) under the z-stack model, and the maximum excitation/emission wavelength was 495/530 nm for egfp and 549/565 nm for DiI, respectively.

Hemolysis test: Red blood cells (RBCs) of a healthy donor were isolated from 1 mL blood (with EDTA as anticoagulant) by centrifugation at 3 000 rpm for 5 min and washed three times with PBS solution (10 mM, 150 mM NaCl, pH 7.2). The purified RBCs were diluted to 10 mL PBS. Then 0.2 mL of diluted RBCs suspension was mixed with 0.8 mL PBS containing different concentrations of 1598 or 1598@mSi. RBCs incubated with nanopure water and PBS was used as the positive and negative control, respectively. All the sample tubes were kept in static condition at 37 ℃ for 8 h. Finally, the mixtures were centrifuged at 3 000 rpm for 5 min, and photographs were taken by a cannon camera.

Procedure for *in vitro* cell viability assay: The cytotoxicity of bacteria to RAW264.7 (immunocyte) and 3T3 (bistiocyte) were tested using standard MTT method. Cells were cultured in Dul-becco's modified Eagle's (DMEM) (Gibco BRL) medium supplemented with 10% fetal bovine serum (FBS) in a 5% CO_2 humidified environment at 37 ℃ for an appropriate time. Then the harvested cells were seeded at a density of 5000 cells/well in 96-well assay plates and allowed to overnight grow at 37 ℃ for attachment. After adding different concentrations of 1598 or 1598@mSi the cells were further incubated for 24 h at 37 ℃ under 5% CO_2. Then the cells were washed with PBS for three times and MTT in fresh DMEM (0.5 mg mL^{-1}, 100 μL) was added. After 4 h incubation at 37 ℃ under 5% CO_2, the supernatant was removed, and the produced formazan was dissolved by DMSO (100 μL per well). Absorbance values of formazan were determined at 490 nm (corrected for background absorbance at 630 nm) with a Bio-Rad model-680 microplate reader. Six replicates were done for

each treatment group. Absorbance values of the samples without cells were considered as controls, and the activities were normalized accordingly.

Bacterial skin infection: BALB/c mice were purchased from Laboratory Animal Center of Jilin University (Changchun, China). All animal experiments were conducted under the guidelines of the Institutional Animal Care and Use Committee. Skin infection experiments were measured by modification of a previously described infection model.[1] Briefly, the hairs on the backs of healthy mice were removed by cream hair remover (Veet). Then 50 μL 0.9% NaCl solution (or containing 5×10^8 CFU mL^{-1} 1598, 1598@mSi) was injected subcutaneously into the back skin. After that, photographs of the skins changes were taken at intervals and the mice body weights were recorded as well. 15 days later, one typical mouse from each of the NaCl injection group and 1598@mSi injection group were sacrificed for histology analysis. Slices of main organs were prepared and stained with hematoxylin and eosin (H&E). The images of the stained organs were collected on an Olympus BX-51 optical system.

Arsenic detection in water: In a typical test, 2 mL water sample was mixed with 0.25 mL 9×LB and 0.25 mL 1598@mSi or 1598 (5×10^8 CFU mL^{-1}) LB solution. Then the mixture was kept at 30 ℃ for 5 h. After that, 1598@mSi were separated and resuspended in pure water for fluorescence test. Fluorescence spectra were measured with a JASCO FP-6500 spectrofluorometer. The standard curve was obtained by adding different concentrations of sodium arsenite to the water samples.

Serum arsenic detection: In a typical test, 0.4 mL serum (fetal bovine) was mixed with 0.1 mL 1598@mSi or 1598 (2.5×10^8 CFU mL^{-1}) LB solution. Then the mixture was kept at 30 ℃ for 5 h. After that, 1598@mSi were separated and resuspended in pure water for fluorescence test. The standard curve was obtained by adding different concentrations of sodium arsenite to the serums.

***In vivo* arsenic detection:** The hairs on the backs of healthy mice (BALB/c) were firstly removed very carefully by cream hair remover (Veet). Then the mice were exposed by subcutaneous injection of sodium arsenite at five points with final concentrations of 336 (1/50 LD50), 840 (1/20 LD50) and 3360 (1/5 LD50) μg kg^{-1}, respectively. Subsequently 50 μL of 1598@mSi (5×10^8 CFU mL^{-1}) was subcutaneously injected at each of the back. 5 h later, fluorescence imaging was performed on a Maestro *in vivo* optical imaging system (Cambridge Research & instrumentation, Inc) with blue light (445-490 nm) excitation.

Supplemental Results and Discussion

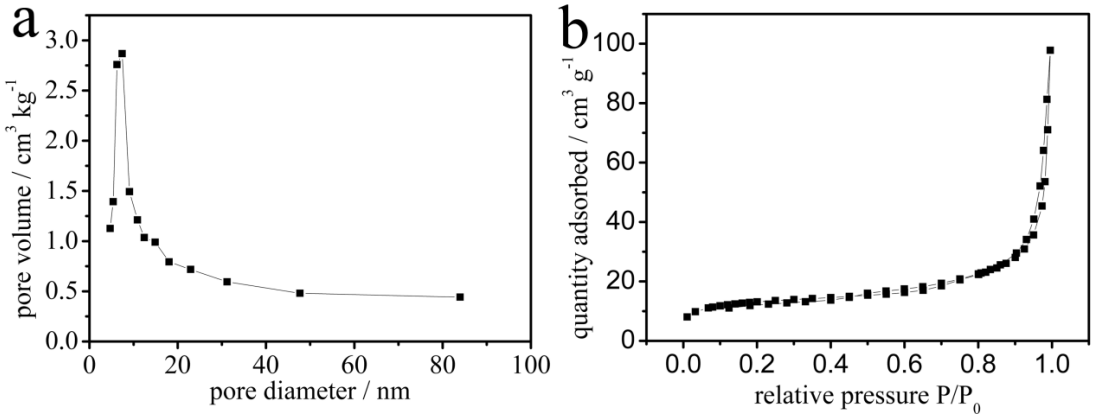

Fig. S1 BJH pore size distribution derived from the adsorption branch of the adsorption isotherm (a) and N_2 adsorption-desorption isotherms (b) of the calcined DH5α@mSi. N_2 adsorption-desorption isotherms were recorded on a Micromeritics ASAP 2020M automated sorption analyzer.

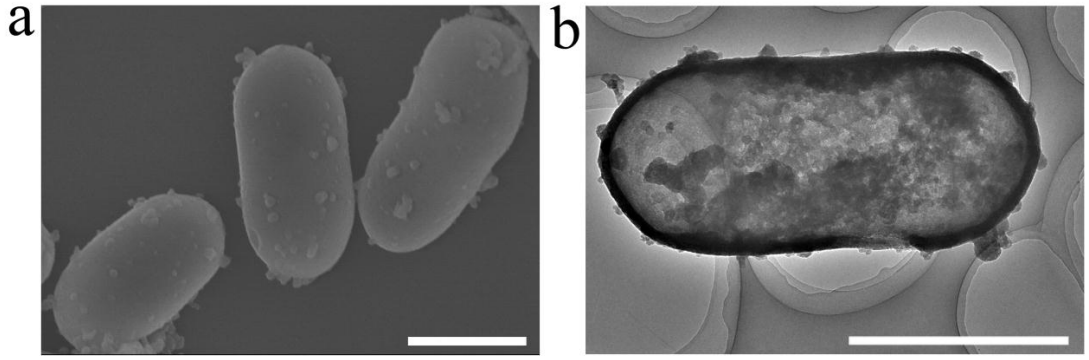

Fig. S2 SEM (a) and TEM (b) images of DH5α@Si, Scale bar = 1 μm. SEM image was obtained with a Hitachi-4800 FE-SEM. TEM image was recorded using a FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV.

Both in our developed method (bacteria@mSi) and the contrast method (bacteria@Si) the same silicification regents TEOS and APTES were used. The primary difference between them is the encapsulation environment. DH5α@mSi was encapsulated in saccharose isotonic solution with biocompatible nonionic surfactant Pluronic®F-127, while the DH5α@Si was fabricated in pure water without Pluronic®F127. There is no obviously contrast in shape between this two type encapsulation from the SEM and TEM images (Fig.1 and Fig. S2). However the two

encapsulated bacteria presented very different livability (Fig. 2) and activity (Fig. S4). Encapsulated through our developed method the bacterial livability and activity were greatly kept. However the bacteria encapsulated through the contrast method showed low livability and little activity.

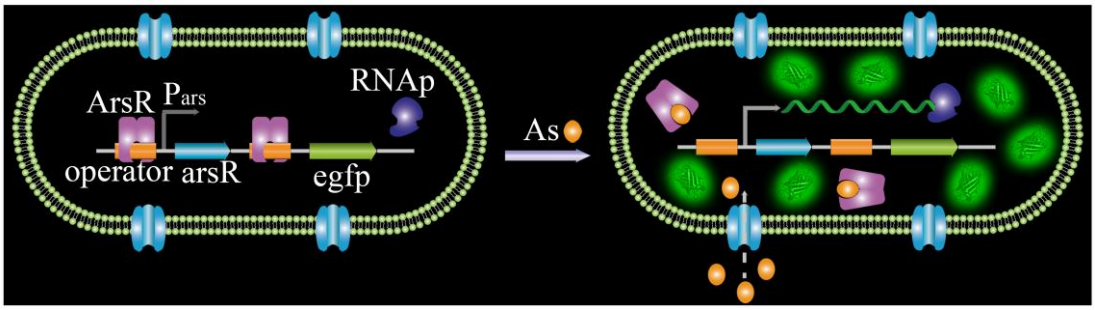

Fig. S3 Outline of the working principle of bioreporter bacteria 1598.

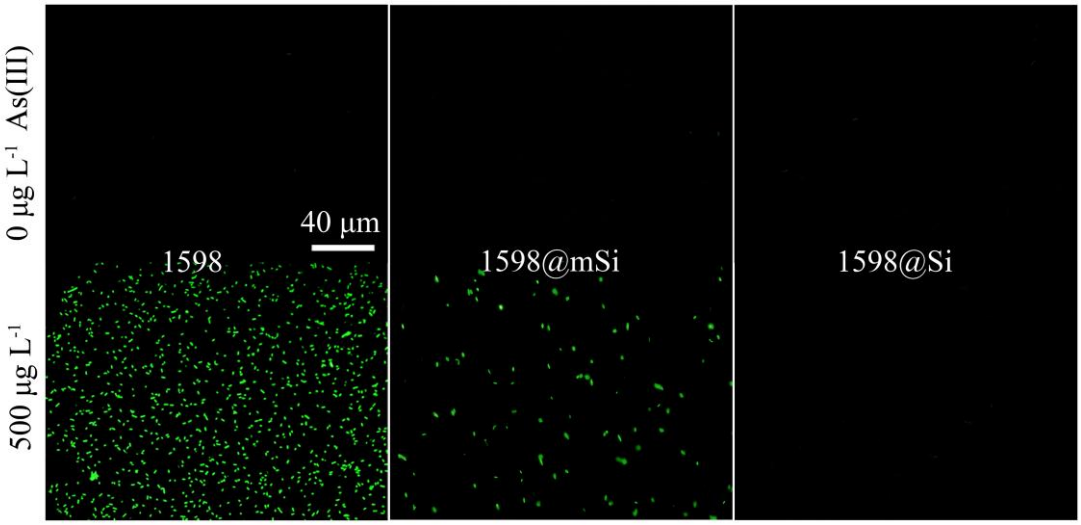

Fig. S4 The fluorescent images of bare 1598, 1598@mSi and 1598@Si induced by 0 or 500 μg L^{-1} of sodium arsenite.

In terms of the working principle of 1598, arsenite ion uptake and protein expression are considered to be the two main factors to influence the detection sensitivity. Arsenite is taken into cells by aquaglyceroporin channels. Protein expression activity is related to the growth condition and growth cycle. Commonly, a rich nutrition condition would promote bacterial protein expression, and in the

proliferative stage bacteria will present a high protein expression activity. Thus, due to the fast arsenite ion and nutrition uptake ability as well as the proliferation ability, bare 1598 showed the highest sensitivity compared with the two encapsulated 1598 (Fig. S4). The restricted proliferation ability (Fig. S5) by the encapsulated layer would be the main reasons for the decreased arsenite responsive activity of 1598@mSi. Although the detection sensitive of 1598@mSi decreased compared with that of bare 1598, in Fig. 5 we have demonstrated that armoured bacteria are still sensitive enough for arsenic contamination samples detection. For the 1598@Si, bacteria arsenite responsive activity was found to be completely inhibited. We consider that except for the limited nutrition uptake (Fig. S6) and proliferation (Fig S5) ability, the probable blocking of arsenic uptake ion channel by the silica shell would also contribute to inhibited biosensing activity of 1598@Si.

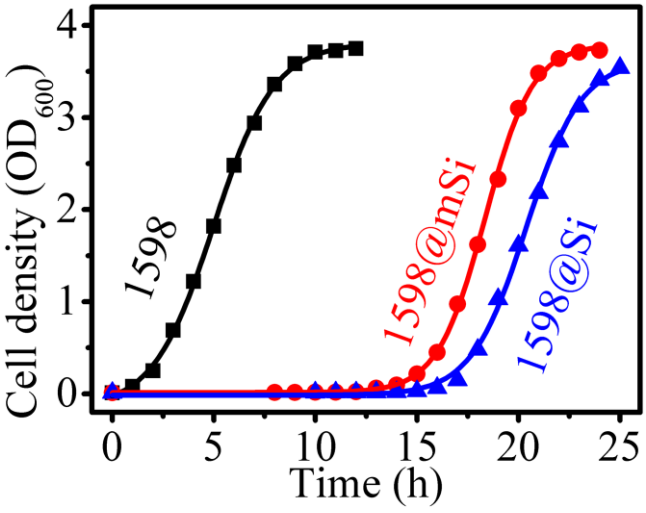

Fig. S5 Growth curves of bare 1598, 1598@mSi and 1598@Si in LB culture medium at 37 ℃.

As seen in Fig. S5, the presented long lag phase in the growth curve of 1598@mSi demonstrates the strong bacteria proliferation inhibitive behavior of mSi shell. After

the long lag phase, the log growth phase similar with that of bare 1598 was also observed in the growth curve of the armoured bacteria 1598@mSi and 1598@Si. This is a common proliferation behavior in almost all previously reported encapsulated bacteria. However, so far we know little about the mechanism. The restrictive force from the encapsulated layer is considered to responsible for the long lag phase. We consider the undesired proliferation of the encapsulated bacteria after the long lag phase may be ascribed to the proliferation of few defectively encapsulated bacteria.

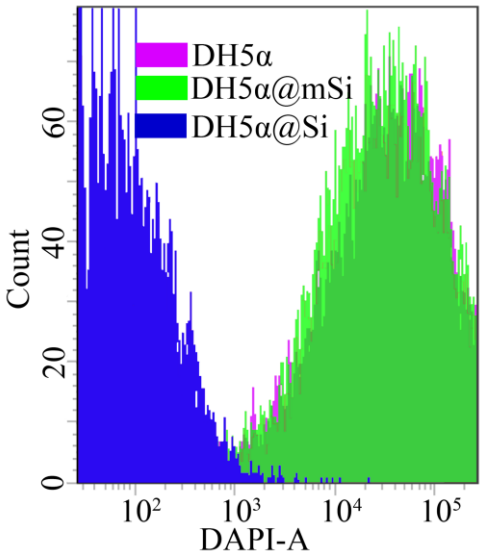

Fig. S6 Flow cytometry of DH5, DH5α@mSi and DH5α@Si stained by DAPI.

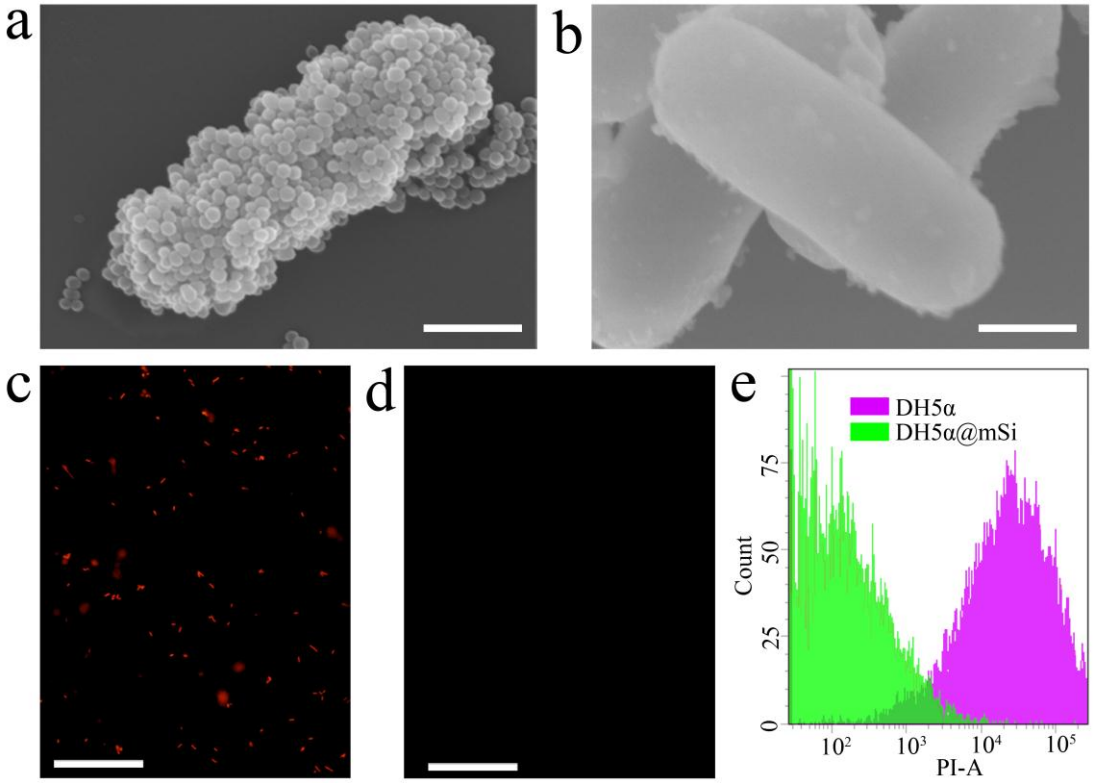

Fig. S7 SEM and fluorescence images of DH5α (a, c) and DH5α@mSi (b, d) after adsorption of SiO_2/R6G@Lys. Flow cytometry of DH5α and DH5α@mSi adsorbed by SiO_2/R6G@Lys (Fluorescence intensity was collect in the PI channel) (e). Scale bar = 1 μm for a and b, 40 μm for c and d.

The select permeability of our fabricated armours was studied through flow cytometry using 4', 6-diamidino-2-phenylindole (DAPI) as small molecule model and lysozyme (Lys) immobilized fluorescent silica nanoparticles (SiO_2/R6G@Lys, Fig. S8a) as virus mimics. DAPI is a blue-fluorescent DNA binding agent which exhibits great enhancement of fluorescence upon binding to dsDNA. Lys is a kind of glycoside hydrolase characterized by the strong affinity with bacteria as well as the ability to damage bacterial cell wall.

In the DAPI permeability test, 1 μL of DAPI stock solution (1 mg mL^{-1} in deionized water) were added to 1 mL pH 7.4 PBS buffer containing bacteria (DH5α, DH5α@mSi or DH5α@Si) with an optical density of 0.2 at 600 nm. After incubation

30 min at room temperature, the bacteria were separated by centrifugation. Then washed three times and resuspended in PBS for flow cytometry test. For the SiO_2/R6G@Lys nanoparticles permeability test, the mixture of SiO_2/R6G@Lys (2 mg mL^{-1}) and bacteria (OD 0.2 at 600 nm) were oscillated at room temperature for 30 min. Then bacteria were separated by centrifugation at 3000 rpm for 2 min and resuspended. The suspension was characterized by flow cytomety and fluorescence microscope.

As shown in Fig. S6, after incubation with DAPI DH5α@mSi displayed a strong fluorescence comparable to that of bare DH5α. However, DH5α@mSi exhibited a much lower fluorescence than that of bare DH5α after incubation with the virus mimics. The SEM and fluorescent images in Fig. S7 also certified that the virus mimics can only bind to the bare bacterial surface, but rarely bind to the armoured bacteria. The porous structure of the mSi shell was considered to be responsible for this select permeability.

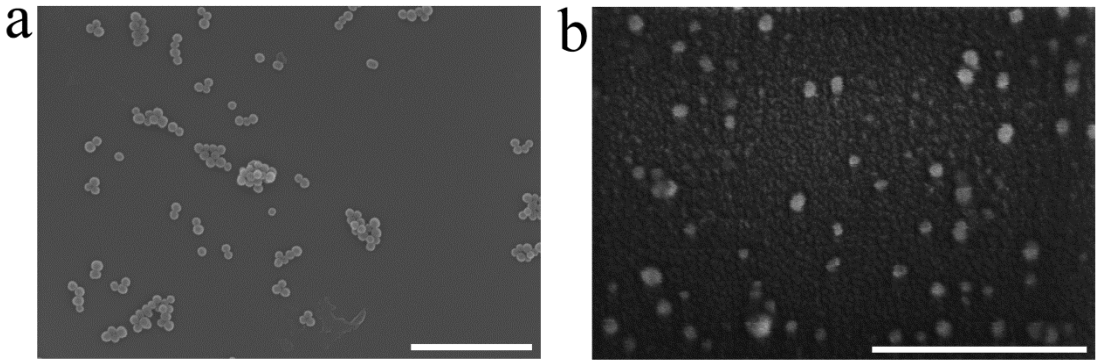

Fig. S8 SEM images of a) SiO_2/R6G@Lys and b) AgNPs. Scale bar = 1 μm and 300 nm respectively.

SiO_2/R6G@Lys was synthesized through several steps. Rhodamine 6G doped silica nanoparticles were firstly synthesized through sol-gel method. Briefly, a mixture of

80 mL ethanol, 4.85 mL H_2O, 3.6 mL $NH_3 \cdot H_2O$ and 5 mg R6G was heated to 55 ℃. Subsequently a mixture of 8 mL ethanol and 3.1 mL TEOS was poured into the mixture. After reaction for 4 h, the nanoparticles were separated through centrifugation at 12000 rpm for 5 min. After washing three times with enthanol, the R6G doped silica nanoparticles (SiO_2/R6G) were resuspended in 100 mL enthanol. Then amino-functionalized SiO_2/R6G (SiO_2/R6G-NH_2) were obtained by srirring with 0.5 mL APTES overnight. Finally the Lys was covalently modified on SiO_2/R6G-NH_2 surface using glutaraldehyde as crosslinking agent. Briefly, SiO_2/R6G-NH_2 ethanol solution was dropped into 5% glutaraldehyde ethanol solution under strong stirring. After reation for 3 h 100 mg $NaBH_3CN$ was added and stirred for another 2 h before washing. The product was resuspended in 50 mL aqueous solution, then a mixture of equal mass of Lys and $NaBH_3CN$ was added. After an overnight reation, the final product SiO_2/R6G@Lys was obtained and separated through centrifugation.

Silver nanoparticles (AgNPs) were prepared by reducing $AgNO_3$ aqueous in the presence of $NaBH_4$. Briefly, 1.2 mL of 80 mM trisodium citrate and 1.5 mL of 20 mM $AgNO_3$ was mixed in flask containing 26.7 mL H_2O. Under strong stirring, 0.6 mL of 100 mM $NaBH_4$ solution was dropped. After stirring for 30 min the solution was aged for at least 24 h to completely decompose the residual $NaBH_4$ before use.

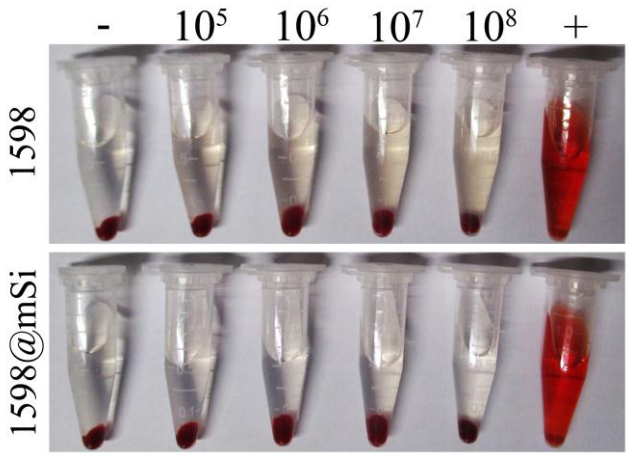

Fig. S9 a) Bacteria induced hemolysis of red blood cells, '-' and '+' represent negative and positive control, respectively.

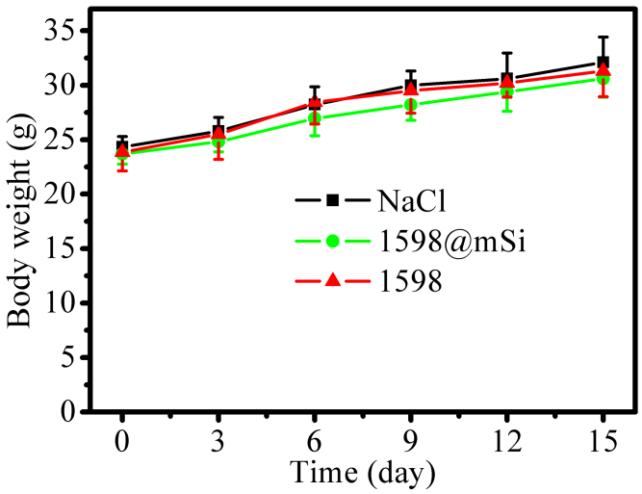

Fig. S10 Change in body weight of mice after being subcutaneously injected with 50 μL of 0.9 wt% NaCl, 1598 and 1598@mSi, respectively.

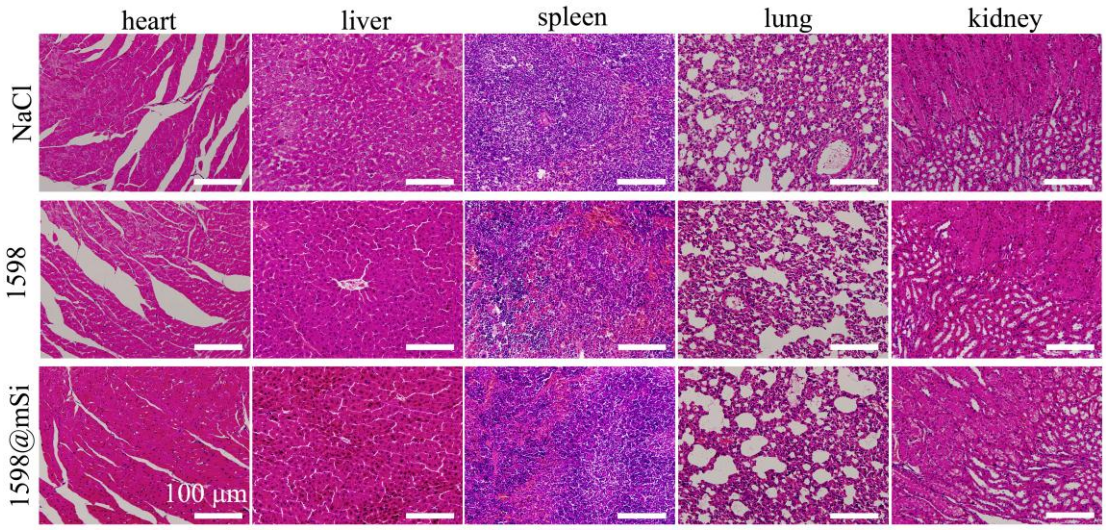

Fig. S11 Histologic sections of the organs from mice on the 15th day after being subcutaneously injected with 50 μL of 0.9 wt% NaCl, 1598 and 1598@mSi, respectively.

The results in Fig. 4 and Fig. S10 and S11 indicated that the virulence of bacteria could be greatly inhibited through this surface chemical engineering. Some reasons were considered to be responsible for the infectivity shielding capacity of the artificial armour. Firstly, the armour blocked the adherence of the bacteria to host cells, which was the critical initial step of bacterial infection.[2] Secondly, the armour would inhibit the bacterial exotoxins being secreted into the extracellular milieu or directly injected into the host cells. Thirdly, the armour could protect bacteria from damage to generate endotoxins. Fourthly, the compact and stable armour restricted fast bacterial proliferation.

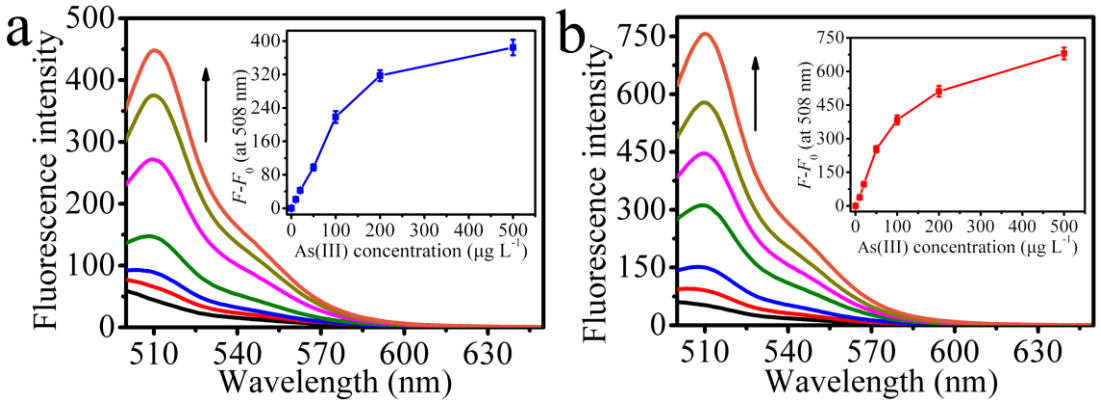

Fig. S12 Fluorescence spectra of arsenic detection in water (a) and serum (b) using bare 1598 as bioreporter, λ_{ex} = 490 nm. The concentrations of the spiked sodium arsenite were 0, 10, 20, 50, 100, 200 and 500 μg L^{-1}, respectively.

Using bare 1598 as bioreporter, the detection limit of spiked arsenic in water and serum was calculated to be 2.5 and 1.6 μg L^{-1} respectively. The values of detection limit of 1598@mSi are 7.1 and 3.4 μg L^{-1} respectively. These results indicated that after being armoured with mSi the arsenic detection sensitivity of 1598 was decreased

which is in consistent with the results observed from Fig. 2. That is to say the artificial armour contributes to improving bacterial vitality and shielding the infectivity, meanwhile it causes a sacrifice of bacterial detection sensitivity. Notably, although the armoured bacteria 1598@mSi showed a little lower sensitive than bare 1598 for arsenic detection, it is still sensitive enough for arsenic contamination samples detection.

Reference

1. B. Muehleisen, D. D. Bikle, C. Aguilera, D. W. Burton, G. L. Sen, L. J. Deftos, R. L. Gallo, PTH/PTHrP and Vitamin D Control Antimicrobial Peptide Expression and Susceptibility to Bacterial Skin Infection. *Sci. Transl. Med.* 2012**, 4**, 135ra66-135ra66.
2. J. Pizarro-Cerdá and P. Cossart, *Cell*, 2006, **124**, 715-727.