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Supporting Information

Nanowire-integrated thermoresponsive microfluidic platform for on-demand enrichment and colorimetric detection of pathogenic bacteria

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Experimental

Materials and instruments

All chemicals were obtained from Sigma-Aldrich and Adamas-beta, and used without further purification. The zeta potential and FTIR spectra were recorded on a nano-sizer (ZS 90, Malvern) and FTIR spectrophotometer (IFS 66/S, Bruker), respectively. The UV-Vis absorption spectra were carried out on a spectrophotometer (UV2450, Shimadzu). The contact angle and TEM experiments were performed by using a drop shape analyzer (DSA 30, Kruss), and a transmission electron microscopy (G20, Tecnai), respectively.

Fabrication of nanowire-integrated thermoresponsive microfluidic chips

The nanowire-integrated thermoresponsive microfluidic chips were fabricated by assembling patterned PNIPAAm-NWs substrates with the chaotic mixing channels with staggered herringbone structures. The patterned silicon nanowire (SiNWs) substrates were produced by simply combining the silver-assisted chemical etching process (Fig. S1) with the standard photolithography.[1] Briefly, a clean n-type silicon wafer with (100) orientation (Thermo Scientific) was immersed into 5% hydrofluoric acid (HF, Sigma-Aldrich) solution to expose the Si-H bonds on the wafer surface. Afterwards, photoresist (AZ 5214) was spin-coated onto a silicon wafer with 100 μm thickness. After exposure of UV light, the silicon wafer was soaked in a 5 mM silver nitrate (Sigma-Aldrich) solution to deposit silver particles on its surface, and the silver particles would act as catalyst in the following etching process. The wafer with silver particles was then soaked in the etching solution containing 4.8 M HF and 0.2 M hydrogen peroxide (H_2O_2, Sigma-Aldrich) at 50 °C for 5 min to produce SiNW arrays. The diameter of SiNWs obtained was in the range of 100-250 nm, while the wire length was around 8 μm. After etching, the SiNWs substrate was immersed in boiling aqua regia (3:1 (v/v) HCl/HNO_3) for 30 min to remove the silver particles on its surface. The patterned photoresist on the silicon substrate was removed by rinsing with acetone and ethanol. Finally, the substrate was rinsed with DI water, dried under nitrogen and ready for surface modification.

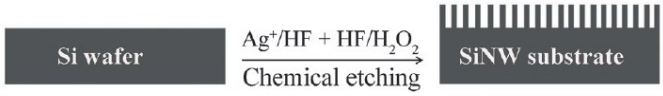

Fig. S1 A schematic of the SiNWs substrate fabrication based on silver-assisted chemical etching.

PNIPAAm-NW substrate was prepared based on the surface-initiated atom-transfer radical polymerization (ATRP) of PNIPAAm on obtained silicon nanowire arrays (Fig. S2). In the surface-initiated ATRP processes, freshly prepared patterned silicon nanowire substrates were firstly refluxed for 6 h in anhydrous toluene containing 5 wt% of 3-aminopropyltrimethoxysilane, then soaked in anhydrous dichloromethane containing 2% (v/v) pyridine after acetone and toluene rinsing, and finally bromoisobutyryl bromide as polymerization initiator was added dropwise into the solution containing the substrates and incubated for 12 h at room temperature. After rinsing with toluene, dichloromethane, methane and deionized water, the substrates were immersed into a degassed solution of the mixture of methanol and deionized water containing copper(I) bromide, N-Isopropylacrylamide, pentamethyldiethylenetriamine, and ethyl 2-bromoisobutyrate, and reacted for 4 h at 30 °C. After washing with deionized water and drying with nitrogen flow, the PNIPAAm-NWs substrates were obtained. The chaotic mixing channels with staggered herringbone structures were purchased from Suzhou Wenhao Chip Technologh Co., Ltd., and assembled with patterned PNIPAAm-NWs substrates obtained above by a homemade holder to fabricate the nanowire-integrated thermoresponsive microfluidic chips.

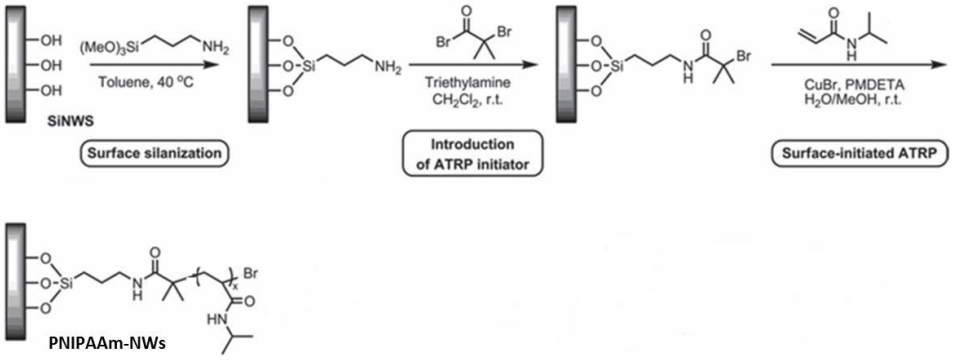

Fig. S2 A schematic of the surface-initiated ATRP to prepare PNIPAAm-NW substrate.

Bacteria culture

Bacteria of *Escherichia coli* (*E. coli*) (ATCC 8739), *Staphyloccus aureus* (*S. aureus*) (ATCC 6538), *Pesudomonas aeruginosa* (*P. aeruginosa*) (ATCC 9027), and *Bacillus. subtilis* (*B. subtilis*) (ATCC 6051) were used in our experiments. Prior to experiments, the bacteria were grown overnight in Luria-Bertani broth medium (LB, Sigma-Aldrich) and harvested at the exponential growth phase via centrifugation. The supernatant was then discarded and the cell pellet was resuspended in different mediums including PBS buffer, DMEM, and blood. The bacteria concentration could be monitored photometrically by measuring the optical density (OD) at a wavelength of 600 nm. Before performing bacteria enrichment and colorimetric experiments, the OD_{600} values of bacteria stock solutions were re-adjusted to 0.1, which corresponded to the concentrations, obtained based on colony counting method, of 5×10^8, 4×10^8, 4×10^8, and 2.5×10^8 CFU/mL for *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. subtilis*, respectively.

Nanowire-integrated thermoresponsive microfluidic chips-based bacterial enrichment and release

Before thermoresponsive bacterial enrichment and release, a further surface modification was carried out for the nanowire-integrated thermoresponsive microfluidic chips. Briefly, hydrophobic anchor (biotin-BSA) solution was firstly pumped into the microfluidic chip and incubated for 1 h at 37 °C. After rinsing with pre-warmed (37 °C) PBS buffer, linker (streptavidin) and bacteria-binding molecules (biotin-con A or biotin-Apt, 5'-HOOC-GCTAACCCCCCCAGTCCGTCCTCCCAGCCTCACACCGCCA-3') solutions were sequentially loaded into the microfluidic chip and incubated for 1 h at 37 °C. After rinsing with pre-warmed (37 °C) PBS, the microfluidic chip was loaded 1 mL of bacterial sample at various flow rates ranged from 0.5 to 5 mL/h at 37 °C to investigate its performance for bacterial enrichment, and the corresponding bacterial enrichment efficiencies defined as the proportion of bacteria captured from the test sample were calculated and analyzed. For thermoresponsive bacterial release, the microfluidic chip with enriched bacteria was put on a heating plate with 25 °C for 30 min, and the corresponding bacterial release efficiencies defined as the proportion of bacteria released from the microfluidic chip were calculated and analyzed. In our experiments, the enrichment efficiency is calculated by comparing the numbers of bacteria in the test sample before and after enrichment determined based on the standard colony counting method, while the release efficiency is calculated by comparing the numbers of bacteria enriched by the microfluidic chip before and after release determined by the standard colony counting method.

Preparation of azide- and alkyne-AuNPs

AuNPs were synthesized by the classic citrate reduction method.[2,3] Briefly, 2 mL of 1% gold(III) chloride trihydrate solution was added into 200 mL of double-distilled water and heated under reflux until boiling. Then, 5 mL of 1% trisodium citrate solution were added under vigorous stirring. Boiling was continued for 15 min to obtain the AuNPs with size of 14 nm. For azide and alkyne groups functionalization, AuNPs were first modified by HS-PEG (MW = 350) and 11-mercaptoundecanoic acid (11-MUA) (HS-PEG : 11-MUA : AuNPs = 1500:1000:1) to obtain the PEG-AuNPs-COOH through ligand-exchange reactions,[4] and then 11-azido-3,6,9-trioxaundecan-1-amine technical and propargylamine (11-azido-3,6,9-trioxaundecan-1-amine technical/propargylamine : 11-MUA = 5:1) were conjugated with the PEG-AuNPs-COOH through standard EDC/NHS chemical covalent coupling procedures,[5] to obtain the azide- and alkyne-AuNPs, respectively.

Highly sensitive detection of pathogenic bacteria based on nanowire-integrated thermoresponsive microfluidic chip and click reaction-assisted colorimetric assays

To prepare the click reaction-assisted colorimetric system for bacterial detection, the azide- and alkyne-AuNPs solutions with same concentrations of 2.7 nM were first mixed, and then $CuCl_2$ solution (100 nM) was added as the copper-ion source for subsequent bacteria reduction and click reaction. For the detection of given bacterial samples, they was first enriched by the nanowire-integrated thermoresponsive microfluidic chip at 37 °C, and then click reaction-assisted colorimetric systems (the mixture of Cu^{2+}, azide-, and alkyne-AuNPs water solutions) were spiked into the chip, incubated with enriched bacteria for 1 h at 25 °C, and finally pumped to microplate wells. The output click reaction-assisted colorimetric systems in microplate wells were then imaged, and the corresponding B/R values of obtained images were calculated by the Adobe Photoshop software to quantify the color changes of the colorimetric systems.

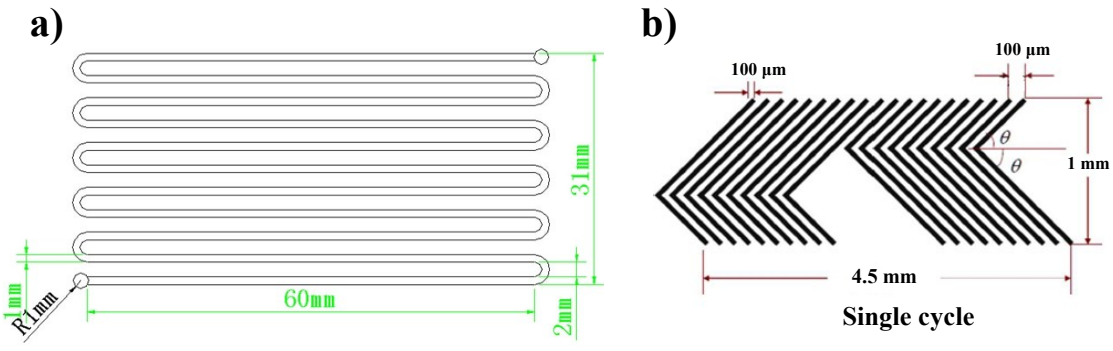

Fig. S3 (a) Design sketch of the chaotic mixing channel with staggered herringbone structures used in our experiments. (b) The detailed parameters of the staggered herringbone structures shown in (a).

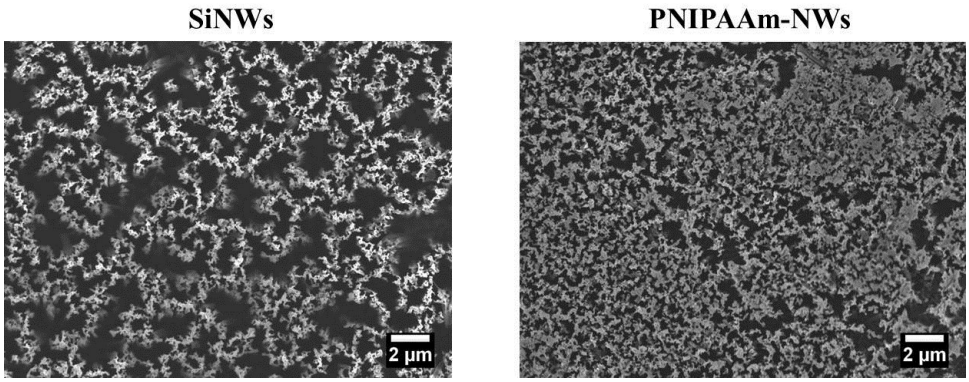

Fig. S4 The SEM images of silicon nanowires (SiNWs) before and after PNIPAAm polymer brush modification (PNIPAAm-NWs).

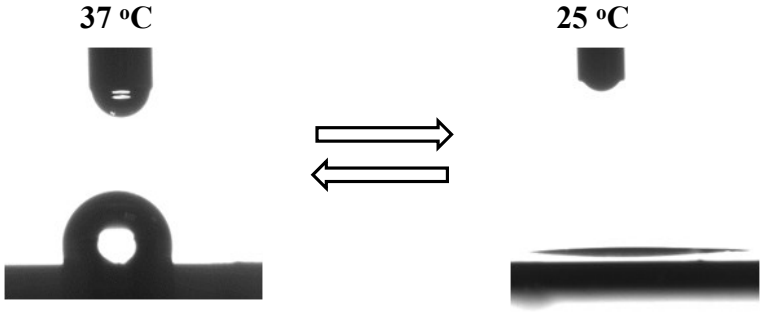

Fig. S5 The contact angle measurement of PNIPAAm-NW substrate at 37 and 25 °C.

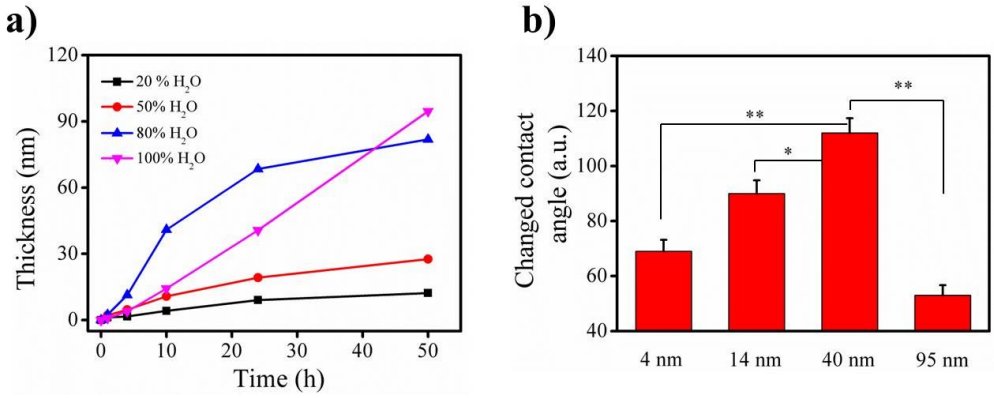

Fig. S6 (a) The thickness of PNIPAAm brush on the surface nanowire substrate vs polymerization time of ATRP reaction in MeOH/H_2O solvent with different water ratios. (b) The effect of PNIPAAm thickness on the change of contact angle of PNIPAAm-NW substrate under the alternation of temperature from 37 and 25 °C. *P < 0.05, **P< 0.01.

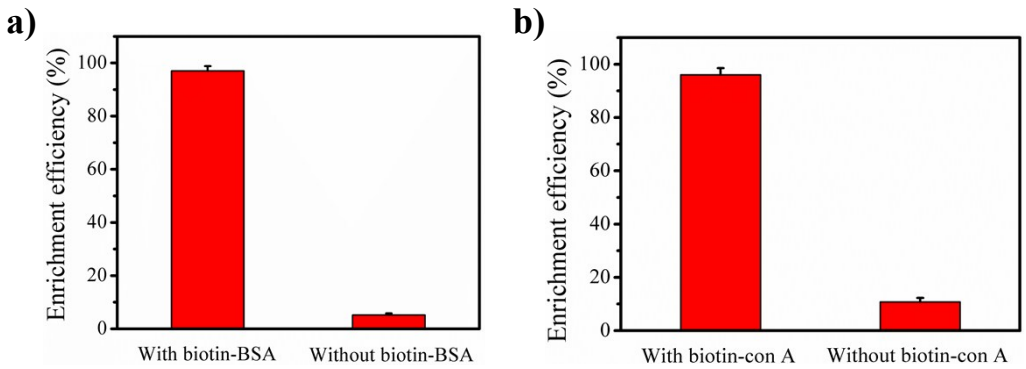

Fig. S7 Quantitative evaluation of the effect of bacteria-binding complexes including biotin-BSA (a) and biotin-con A (b) modification of the PNIPAAm-NW-con A chip on its bacteria-enrichment efficiency. The values of bacterial enrichment efficiency represent the mean of three independent experiments and the error bars indicate the SD from the mean. 10^5 CFU of *E. coli* was used, and the flow rate was 1 mL/h.

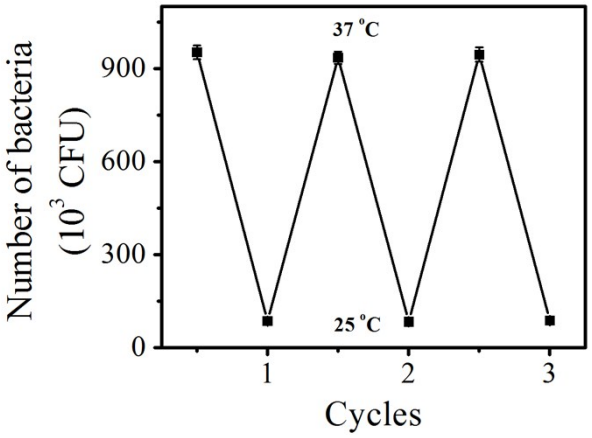

Fig. S8 Quantitative date for reversible capture and release of bacteria on PNIPAAm-NW-Con A chip in response to periodic temperature change from 37 and 25 °C. The separation of 10^6 CFU of *E. coli* was conducted.

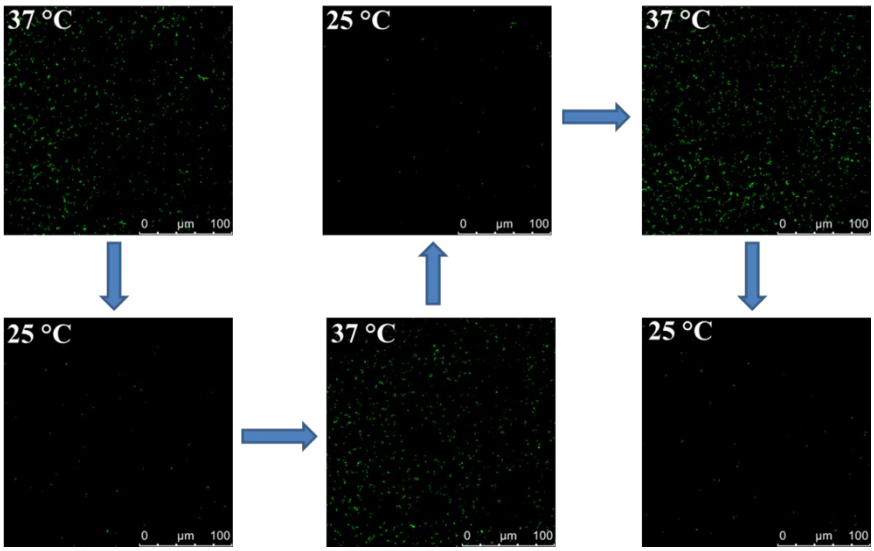

Fig. S9 Representative fluorescence images for reversible capture and release of bacteria on PNIPAAm-NW-Con A chip in response to periodic temperature change from 37 and 25 °C. The separation of 10^6 CFU of *E. coli* was conducted, and the green-fluorescent dye of SYTO 9 was used to stain the *E. coli* bacteria captured.

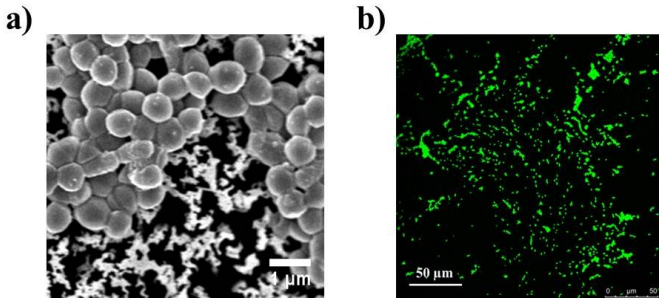

Fig. S10 (a) Representative SEM image of *S. aureus* captured by PNIPAAm-NW-Apt chip. The separation of 10^6 CFU of *S. aureus* was conducted. (b) live/dead staining image of *S. aureus* captured by PNIPAAm-NW-Apt chip. The separation of 10^6 CFU of *S. aureus* was conducted. Two fluorescent dyes were used in live/dead bacterial staining in which SYTO 9, with a green color, labeled live bacteria, while propidium iodide, with a red color, stained only dead bacteria.

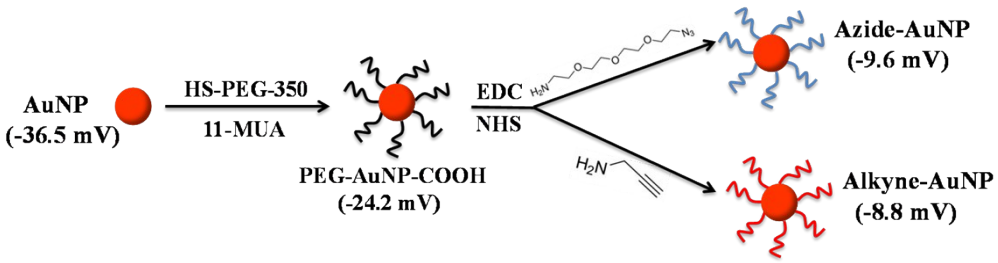

Fig. S11 The preparation processes of azide- and alkyne-AuNPs. The zeta potentials of AuNPs, PEG-AuNP-COOH, azide-, and alkyne-AuNPs were given and used as an effective way to monitor the process of AuNPs functionalization. The zeta potential values represent the mean of three independent detections.

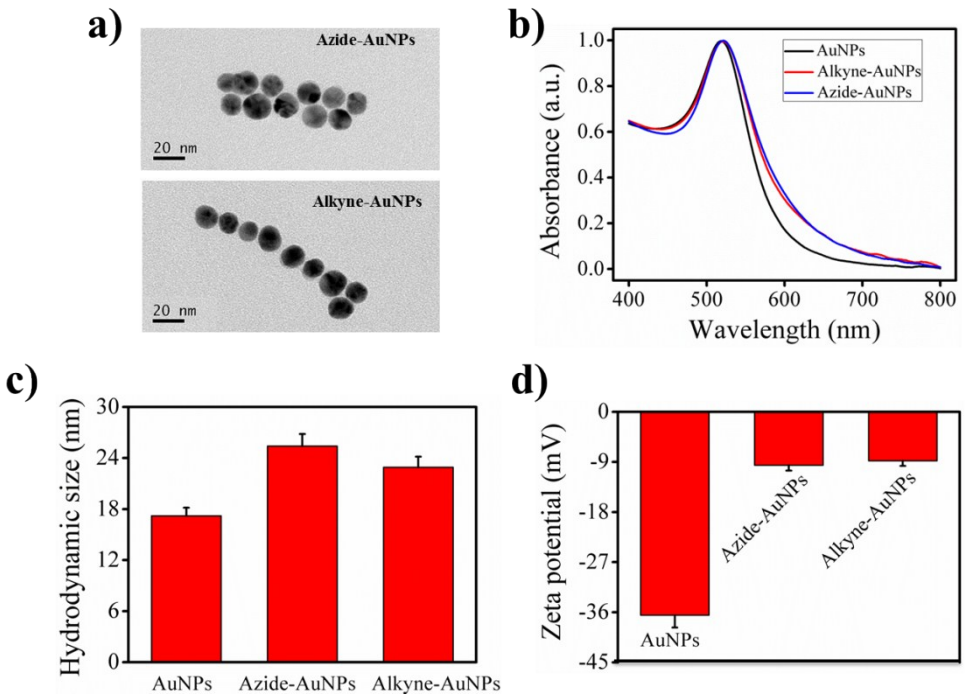

Fig. S12 (a) TEM images of azide-, and alkyne-AuNPs. (b) UV-vis absorption spectra of AuNPs, azide-, and alkyne-AuNPs in DI water. (c) Hydrodynamic sizes of AuNPs, azide-, and alkyne-AuNPs in DI water. (d) Zeta potentials of AuNPs, azide-, and alkyne-AuNPs in DI water. In (c) and (d), the values of hydrodynamic size and zeta potential represent the mean of three independent experiments, and the error bars indicate the standard deviation (SD) from the mean.

Fig. S13 Typical photographs of the click reaction-assisted colorimetric system incubated with different species of pathogenic bacteria enriched by the PNIPAAm-NW-con A chip. The click reaction-assisted colorimetric system without bacteria incubation was used as the control. From left to right: control, *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis*. In the experiments, 1 mL of different species of pathogenic bacteria PBS suspensions (10^3 CFU/mL) were first enriched respectively by the PNIPAAm-NW-con A chip at 37 °C, then click reaction-assisted colorimetric system was spiked into the chip, and finally pumped to microplate well after incubation with the enriched bacteria for 1 h at 25 °C. The output click reaction-assisted colorimetric systems in microplate wells were then imaged.

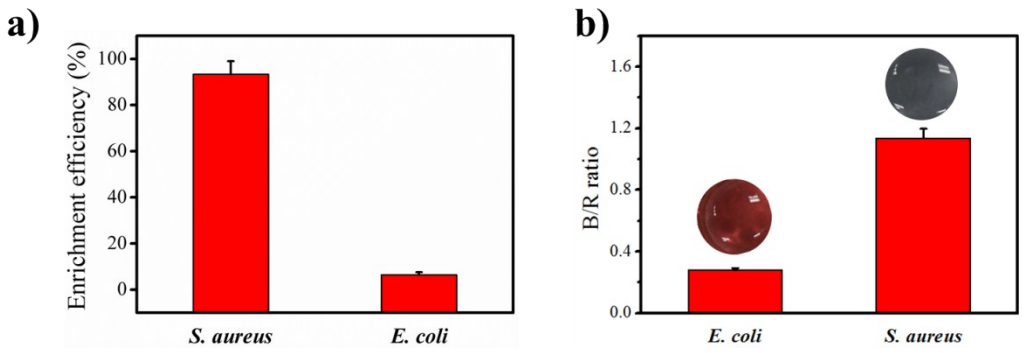

Fig. S14 (a) Quantitative evaluation of enrichment efficiency of PNIPAAm-NW-con A chip for *S. aureus* and *E. coli*, respectively. The values of bacterial enrichment efficiency represent the mean of three independent experiments and the error bars indicate the SD from the mean. The separation of 10^5 CFU of *S. aureus* and *E. coli* was performed, respectively, and the flow rate was 1 mL/h. (b) Typical photographs and corresponding B/R values of the click reaction-assisted colorimetric system incubated with *E. coli* and *S. aureus*, respectively, enriched by the PNIPAAM-NW-Apt chip. In the experiments, 1 mL of *E. coli* and *S. aureus* PBS suspensions (10^5 CFU/mL) were first enriched respectively by the PNIPAAm-NW-Apt chip at 37 °C, then click reaction-assisted colorimetric system was spiked into the chip, and finally pumped to microplate well after incubation with the enriched bacteria for 1 h at 25 °C. The output click reaction-assisted colorimetric systems in microplate wells were then imaged, and the corresponding B/R values of the photographs were calculated by the Adobe Photoshop software. The B/R values represent the mean of nine photographs from three independent experiments and the error bars indicate the SD from the mean.

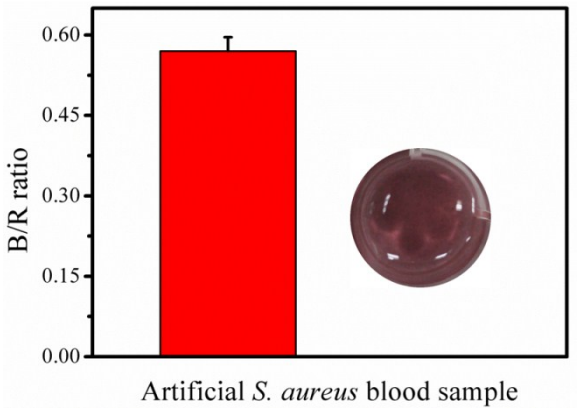

Fig. S15 Typical photograph and corresponding B/R value of the click reaction-assisted colorimetric system incubated with *S. aureus* enriched from the complex artificial blood sample by the PNIPAAM-NW-Apt chip. In the experiments, 10 mL of complex artificial containing 40 CFU/mL of *S. aureus* and 500 CFU/mL of *E. coli* was first enriched by the PNIPAAm-NW-Apt chip at 37 °C, then click reaction-assisted colorimetric system was spiked into the chip, and finally pumped to microplate well after incubation with the enriched *S. aureus* bacteria for 1 h at 25 °C. The output click reaction-assisted colorimetric system in microplate well was then imaged, and the corresponding B/R value of the photograph was calculated by the Adobe Photoshop software. The B/R values represent the mean of nine photographs from three independent experiments and the error bars indicate the SD from the mean.

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