Electronic Supplementary Information (ESI)

Atomic-Engineered Gold@SilverGold Alloy Nanoflowers for *In Vivo* Inhibition of Bacteria

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METHODS

Morphology study of bacteria

SEM images of *E. coli* treatment with Au@AgAu ANFs (10 μg/mL) was obtained based on the previous report.[1] Firstly, *E. coli* cells (10^6 CFU/mL) before and after treatment with Au@AgAu ANFs was dropped onto silicon wafer, which was then treated with 2 % glutaraldehyde fixation for 2 h at room temperature and gradient dehydration by a series of ethanol solutions (50 %, 70 %, 90 %, 95 %, and 100 %) for 10 min with each step. Next, the silicon wafer was subjected to nitrogen drying to maintain the morphologies of the bacteria captured on substrate. After complete drying, the silicon wafer was sputter coated with platinum and imaged with SEM (Hitachi, S-3000N).

TEM images of *E. coli* treatment with Au@AgAu ANFs was obtained based on the previous report.[2] Typically, *E. coli* cells (10^6 CFU/mL) were suspended in 10 μg/mL of NPs and cultured at 37 °C for 60 min. At selected time points (0, 5, 10, 20, 40 and 60 min), *E. coli* cells were collected and fixed with 2.5 % glutaraldehyde for 30 min. Next, cells were washed with 0.9 % NaCl and fixed with 1% aqueous OsO_4 (Fluka) for 1 h, and washed again twice with 0.9 % NaCl. Cells were then dehydrated via ethanol series (70 %, 90 % and 100 % for 15 min, respectively) and embedded in Epon/Araldite resin (polymerization at 65 °C for 15 h). Thin sections (90 nm) containing cells were placed on the grids and stained for 1 min each with 4 % uranyl acetate (1:1 acetone/water) and 0.2 % Raynolds lead citrate (water), air-dried, and examined under the TEM (Hitachi, H-7650).

ICP-MS measurement

The concentration of NPs were measured based on the previous report.[3] Briefly, 300 μL of NPs aqueous suspensions was completely digested with 3 mL of HNO_3 and 1 mL of H_2O_2 in a 100 mL beaker at 130 °C. The solution was evaporated to 300 μL and subsequently diluted to 10 mL using 2% HNO_3. Standard solutions of Au and Ag (1, 5, 10, 50, 100, and 150 ng/mL) were prepared with 2 % HNO_3 and then tested to obtain the standard curves. The amount of Au and Ag in various NPs were measured using inductively coupled plasma mass spectrometry (ICP-MS, Bruker-M90).

Bacteria adhesion test and ICP-MS assay

To quantitative analyze Au@AgAu ANFs adhered on bacterial surface, 1 mL of bacteria suspension (10^6 CFU/mL) were mixted with 1 mL of particle solution (10 μg/mL), which was incubated at 37 °C for 6 h. Then, the solution was filtered through a 0.22 μm pore filter membrane, and washed with PBS thoroughly.[4] The Au@AgAu ANFs adhered on bacterial surface remain on the filter membrane, the filtrate was analyzed by ICP-MS, Au@AgAu ANFs with bacteria-free samples was filtered through 0.22 μm pore filter membrane, the sample was analyzed by ICP-MS as control, the metal amount adhered on bacteria was further calculated based on each bacteria, where the bacteria number was roughly estimated according to its optical density (OD) reading at 600 nm.

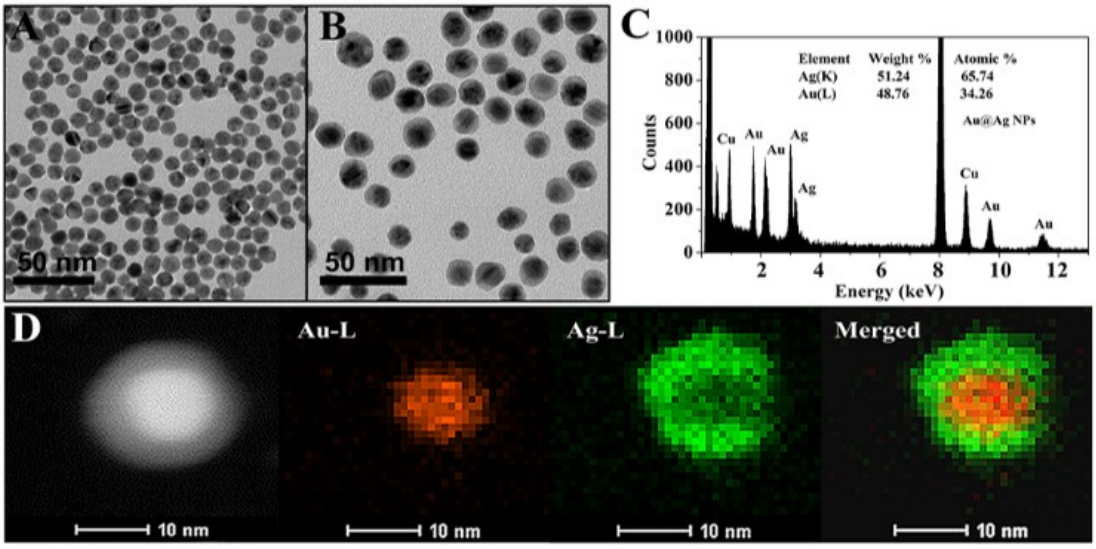

Figure S1. TEM images of (A) Au NPs and (B) Au@Ag NPs. (C) EDX spectra and (D) elemental mapping of Au@Ag NPs.

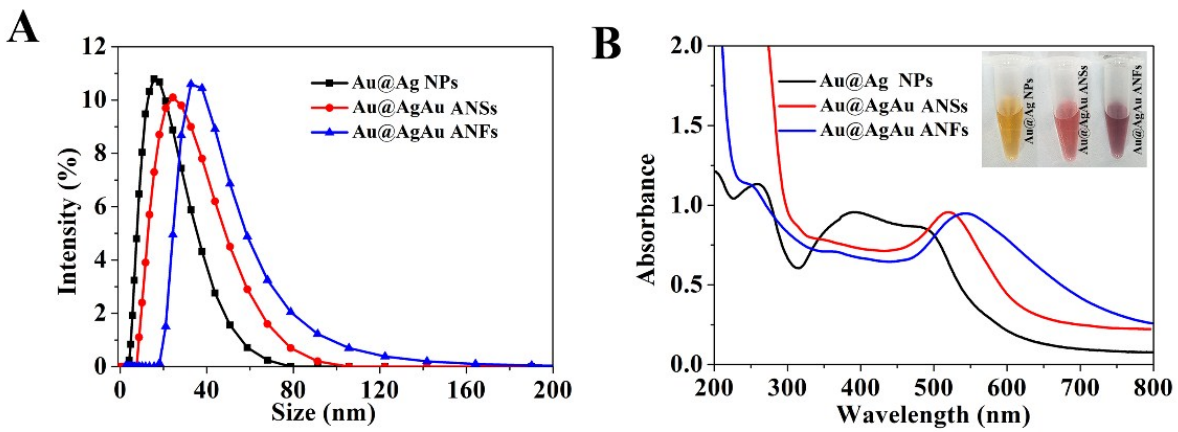

Figure S2. (A) Hydrodynamic diameter and (B) UV-vis spectra of Au@AgAu ANFs.

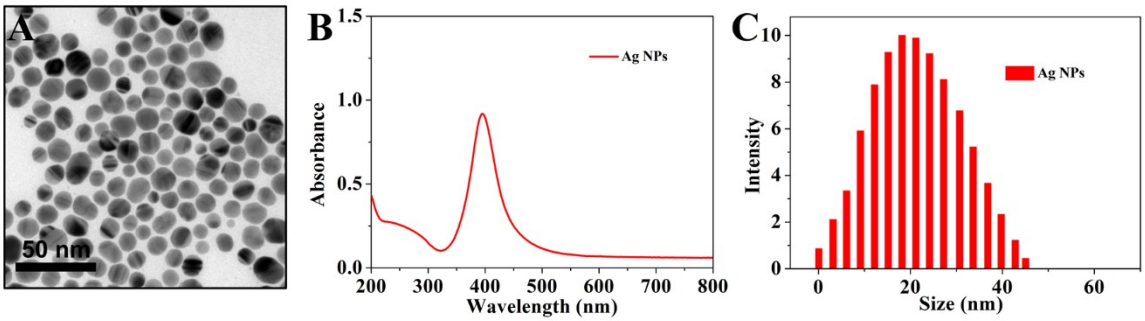

Figure S3. (A)TEM image, (B) UV-vis spectrum and (C) Hydrodynamic diameters of Ag NPs.

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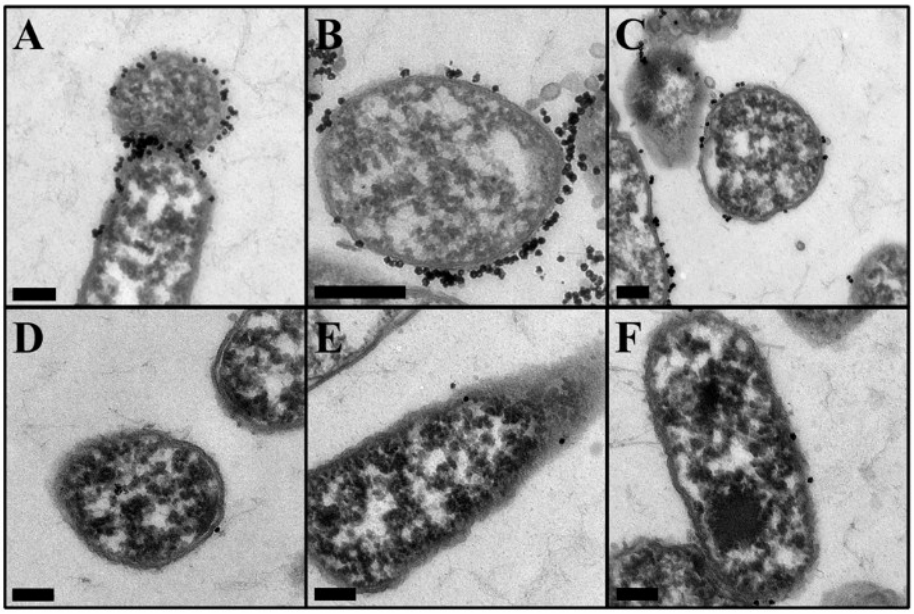

Figure S4. TEM images staining of *E. coli* after treatment with (A-C) Au@AgAu ANFs and (D-F) Au@AgAu ANSs for 6h. The concentration of nanomaterials was 10 μg/mL. Scan bar is 200 nm.

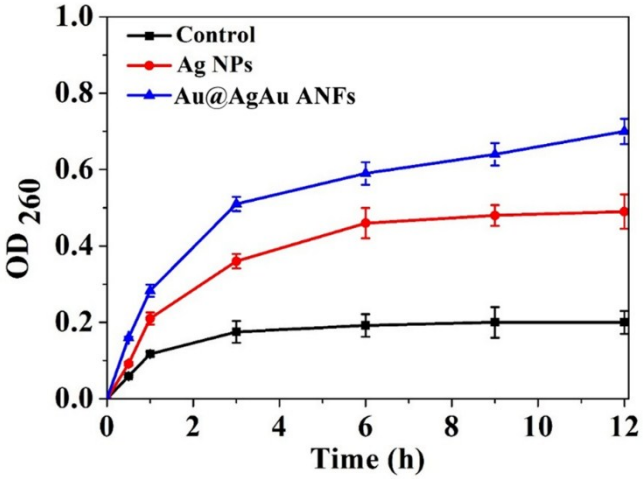

Figure S5. The DNA leakage from *E. coli* after treatment with Ag NPs and Au@AgAu ANFs at different times, respectively.

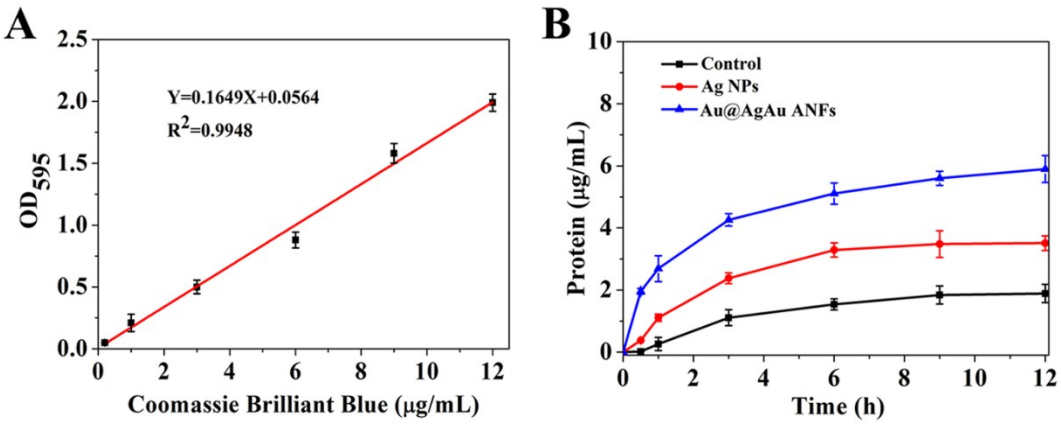

Figure S6. (A) The standard curve of protein measured by coomassie brilliant blue dye binding assay. (B) The protein leakage from *E. coli* after treatment with Ag NPs and Au@AgAu ANFs at different times, respectively.

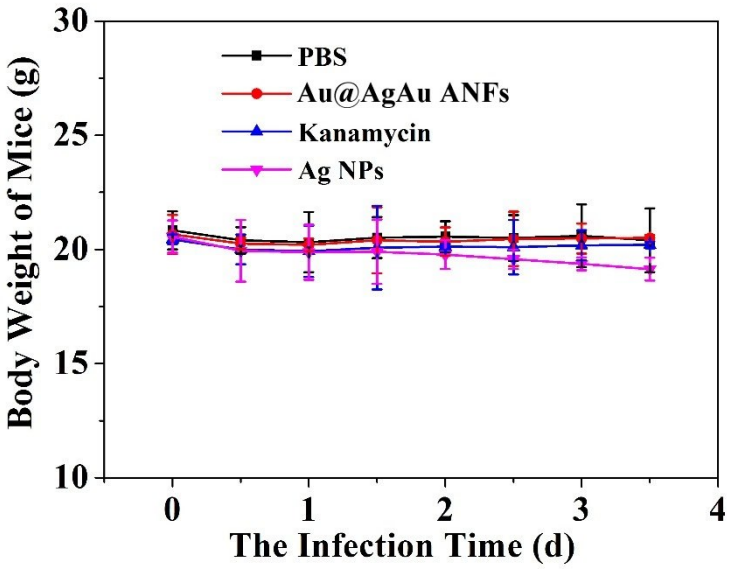

Figure S7. The body weights of mice under different treatments within four days.

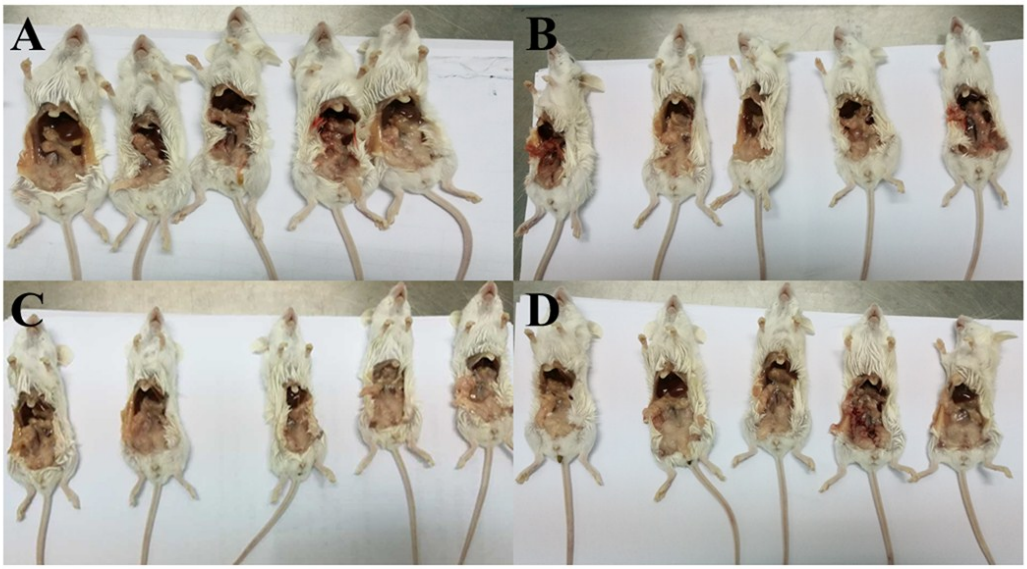

Figure S8. The photographs of intestine infected BALB/c mice. Five groups of mice were sacrificed at fourth day and the intestines were harvested for further analysis. (A) PBS, (B) Au@AgAu ANFs, (C) kanamycin, (D) Ag NPs. The concentration of kanamycin and NPs were 2.5 mg/mL.

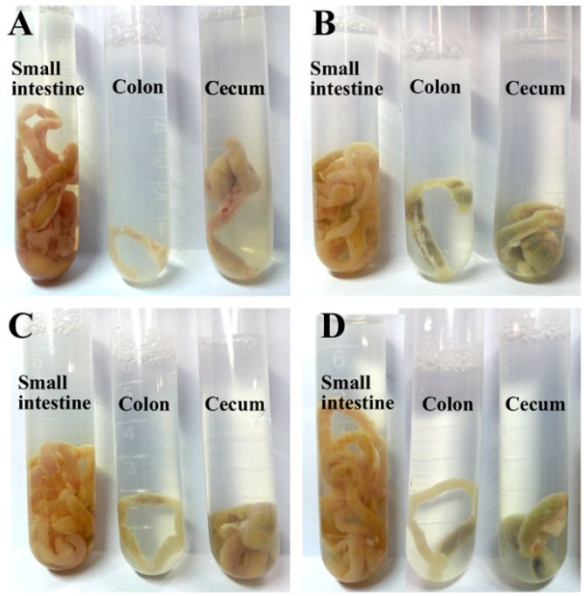

Figure S9. The photograph of small intestine, colon and cecum of the mice infected with *E. coli* and orally ingested by gavage with (A) PBS, (B) Au@AgAu ANFs, (C) kanamycin, (D) Ag NPs twice a day.

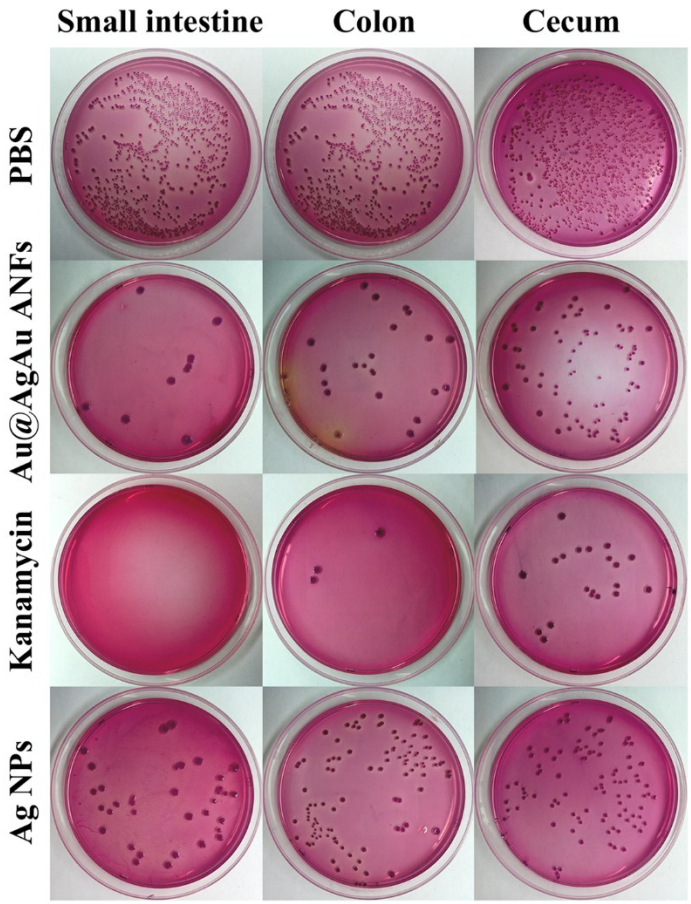

Figure S10. Bacterial colonies in the small intestinal, cecum, and colon after different treatment.

Supplementary Tables

Table S1. ICP-MS results for the samples as prepared

Samples	Au (μg/mL)	Ag (μg/mL)	Au (%)	Ag(%)
Au NPs	115.0	/	100	0
Ag NPs	/	145.0	0	100
Au@Ag NPs	4.0	4.0	50	50
Au@AgAu ANSs	6.9	4.0	63	37
Au@AgAu ANFs	12.7	4.0	76	24

Table S2. Minimum inhibitory concentrations of nanostructures

Bacteria	MIC（μg/mL）				
	Au NPs	**Ag NPs**	**Au@Ag NPs**	**Au@AgAu ANSs**	**Au@AgAu ANFs**
***E. coli* (ATCC25922)**	>500±10	15±2.5	10±1.4	8.9±1.5	4.8±1.0

Reference

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