SUPPORTING INFORMATION

Highly Sensitive and Selective Colorimetric Visualization of Streptomycin in Raw Milk Using Au Nanoparticles Supramolecular Assembly

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1. Materials: $HAuCl_4$, sodium citrate and mercaptoacetic acid were all bought from Alfa&Aesar Company. All other solvents and reagents in this investigation were of analytical grade and used without further purification. All stock solutions were prepared with Milli-Q-purified distilled water.

2. Characterization: The size and the aggregation status of the MPA-stabilized gold nanoparticles were examined by using JEOL JEM-2100F transmission electron microscopy (TEM). UV-Vis spectra were recorded on a Hitachi U-3010 UV-Vis spectrophotometer. Photographs for color changes were taken with a Sony digital camera.

3. Preparation of the MPA-stabilized Gold Nanoparticles: The MPA-stabilized gold nanoparticles were prepared by ligand-exchange reaction between MPA and the citrate-stabilized gold nanoparticles. We synthesized 15-nm gold nanoparticles by the reduction of $HAuCl_4$ by sodium citrate as reported.[1] Ligand-exchange reaction was performed at room temperature by mixing a 100 mL of the as-prepared gold colloids with a 100 μL of aqueous solution of 20 mM MPA under stirring. The mixed solution was then stirred overnight under the room temperature. To improve the performance of the probe, we washed the Au NPs to remove the sodium citrate and excessive MPA. 100 mL of the MPA-stabilized Au NPs solution was centrifuged under 10,000 rpm for 30 min, and supernatant was removed. The MPA-Au NPs were then redispersed by water. The nanoparticle solution was diluted by water to give a total volume of 100 mL (final concentration of gold nanoparticles: 15 nM).[2] MPA-Au NP stock solution is stored under 5 ^{o}C (pH = 5).

4. The effect of pH:

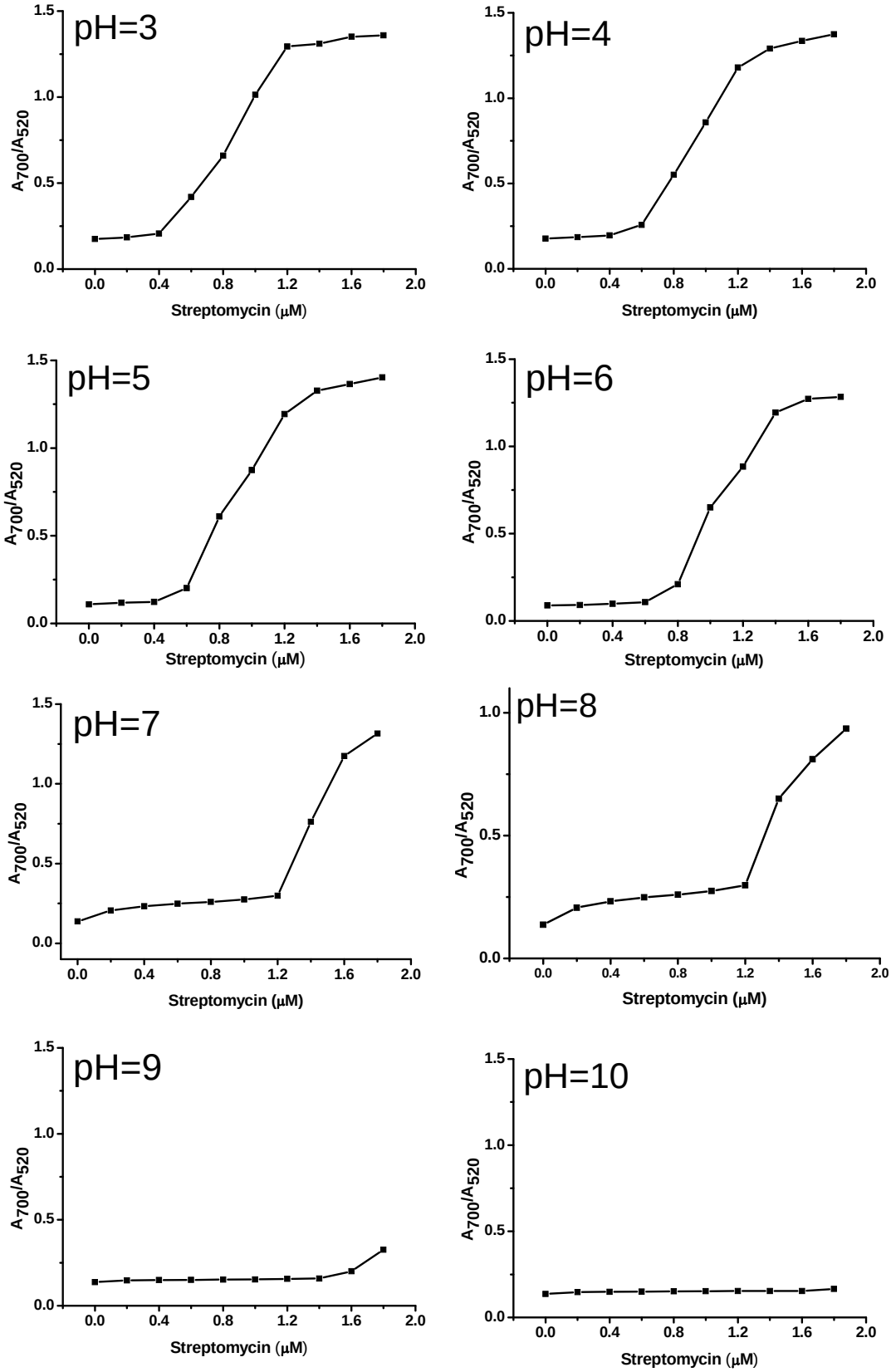

Fig. S1: The plot of ratio A_{700}/A_{520} of MPA-Au NPs versus streptomycin concentrations under different pH conditions (pH = 3-10).

5. MPA-Au NP linear assembly: in order to investigate the effects of streptomycin concentration on Au NP linear assembly, different concentrations of streptomycin were used. Fig. S3 exhibits transmission electron microscopy (TEM) images of the different nanostructures obtained from the addition of different concentrations of streptomycin. When the experiment was carried out in the absence of streptomycin, mostly independent Au nanoparticles were observed on the TEM grid surface (Fig. S2a). This conforms directly to the UV-Vis results, which indicated that the nanoparticles remained independent as the 520 nm plasmon band remained unchanged (shown in Fig. S2). When 0.1 μM of streptomycin was added to MPA-modified Au nanoparticles solution, dimers and trimers were noticed, which was considered as the initiation and building blocks of Au NP chains (Fig. S3b). Clearly, under such low concentration, the number of streptomycin molecules was not enough to induce chain formation. When concentration of the streptomycin was increased to 0.5 μM, short linear chains of Au nanoparticles were formed (Fig. S3c), which indicated that the quantity of streptomycin molecules was too low to pull the Au NPs together and induce long-chain linear assembly.

As the concentration of streptomycin increased to 1 μM, a larger set of linear chains was observed with a degree of branching over random orientations (Fig. S3d). The individual chains were up to several micrometers in length and composed of coaligned single nanoparticles that formed branched superstructures. UV-Vis spectra also showed a corresponding progressive increase of the longitude SPR absorption at 700 nm (shown in Fig. S2). When the streptomycin concentration was up to 4 μM, larger linear structures were observed (Fig. S3e). In these samples, a higher degree of branching was noticed, as well as a degree of nanoparticles aggregation. This is because increasing number of streptomycin molecules can assemble more Au NPs into superstructures. Interestingly, when the streptomycin concentration was increased to 6 μM, chainlike superstructures rarely appeared in the vision of TEM and higher

degree of bulk and random aggregation was observed (Fig. S3f). It suggested that high concentration of streptomycin favored increasing number of branching points, which eventually led to fully reticulated or densely compacted nanoparticle agglomerates. According to the above results, it is concluded that the concentration of streptomycin is crucial in the formation of 1D gold assembly. The guanidyl groups on streptomycin molecules can induce negative charged Au NPs to assemble into 1D structure due to electrostatic interaction between streptomycin and MPA modified-Au NPs.

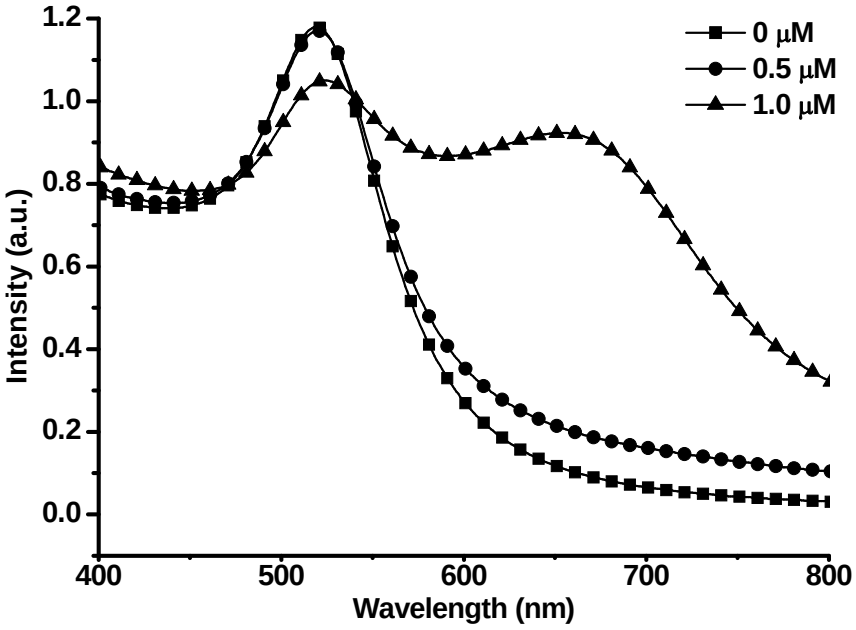

Fig. S2 UV-spectra of addition of streptomycin concentrations of 0 μM, 0.5 μM and 1 μM to MPA-Au NP solution.

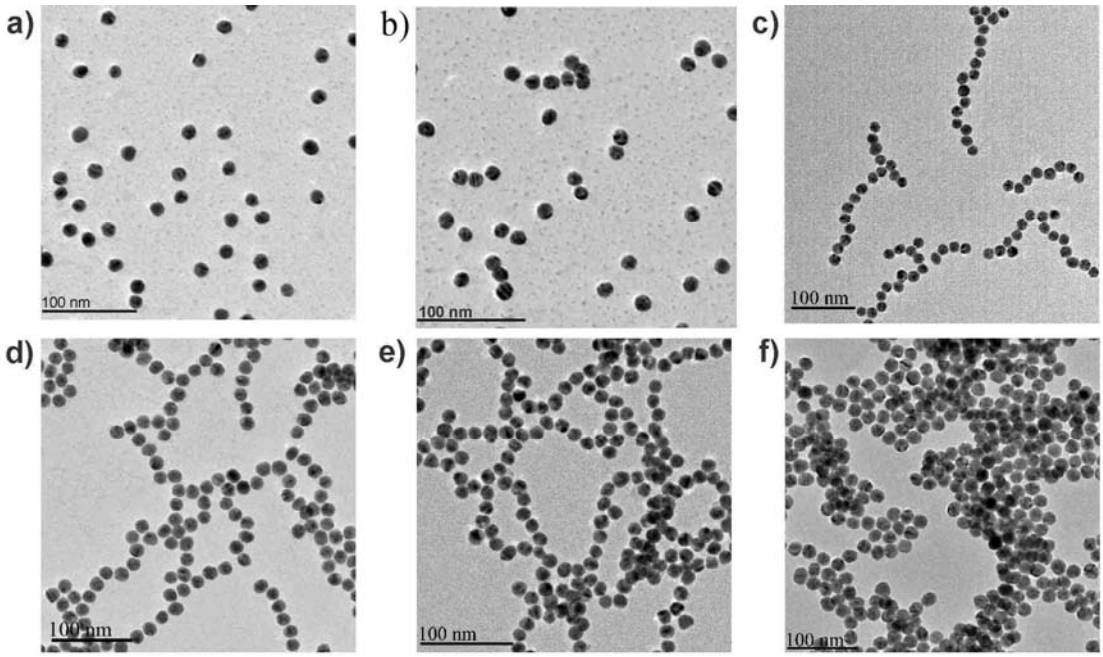

Fig. S3 TEM characterized Au NP aggregation upon addition of streptomycin concentrations up to 0 μM (a), 0.1 μM (b), 0.5 μM (c), 1μM (d), 4 μM (e), and 6 μM (f).

6. The effect of temperature:

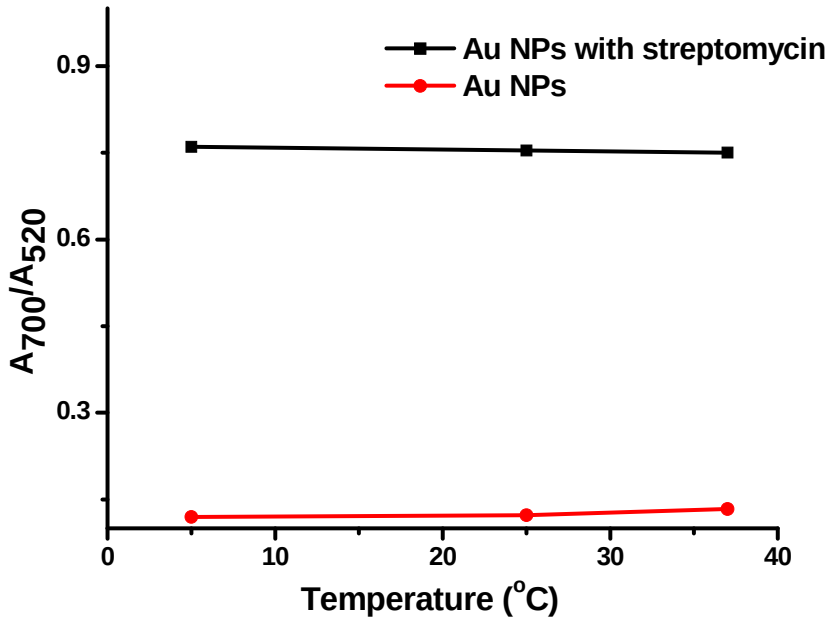

Fig. S4 The plot of ratio A_{700}/A_{520} of MPA-Au NP (red line) and MPA-Au NPs with 1μM streptomycin (black line) under different conditions.

7. Visual colorimetric change of the MPA-stabilized Au NPs upon addition of streptomycin:

Fig. S5 Visual colorimetric change of the MPA-stabilized Au NPs upon addition of streptomycin with different concentrations (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, and 1.8 μM).

8. Optimization of Probe

To improve the sensitivity of the probe, 0.6 μM streptomycin was pre-added to Au NP stock solution. As shown in Fig. S4, the UV-Vis spectrum revealed that the absorption peak of optimized probe at 700 nm increased gradually upon addition of increasing streptomycin concentrations from 80 to 480 nM. Under the optimized condition, linear relationship was found between the A_{700}/A_{520} ratio and concentration of added streptomycin over the range of 80-480 nM (R = 0.991), as shown in inset of Fig. S6. The limit of detection (LOD) at S/N ratio of three for streptomycin was determined to be 2 nM.

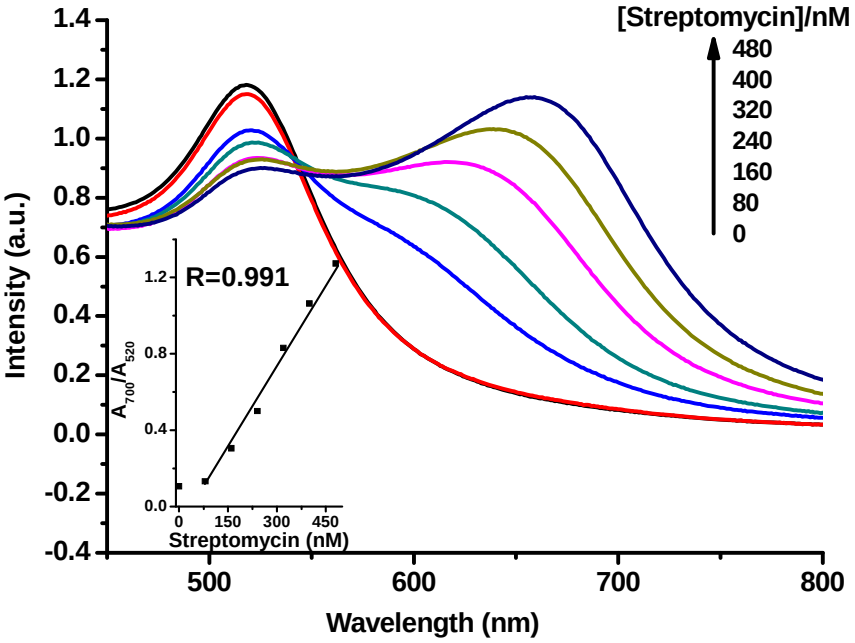

Fig. S6 Concentration-dependent extinction spectra for the MPA-stabilized Au NPs after pre-addition of 0.6 μM streptomycin. Inset: the plot of ratio A_{700}/A_{520} versus streptomycin concentrations.

9. Selectivity Evaluation of the Probe.

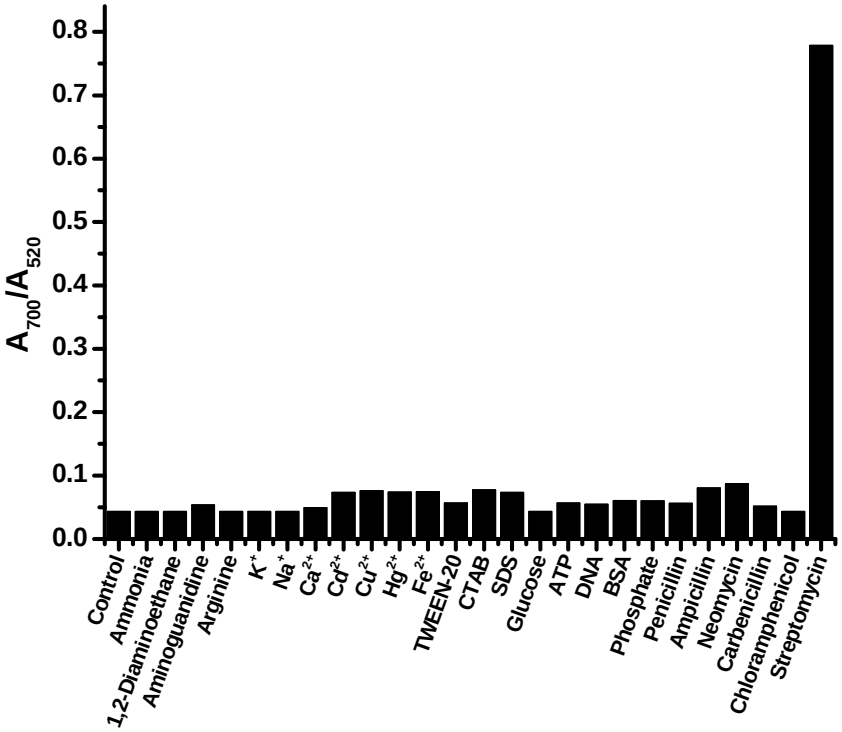

Fig. S7 The selectivity of MPA-Au NPs for antibiotics (1 μM streptomycin, penicillin, ampicillin, neomycin, carbenicillin and chloramphenicol), ions (1 μM KCl, NaCl, $CaCl_2$, $Cd(ClO_4)_2$, $Hg(ClO_4)_2$, $Fe(ClO_4)_2$, $Cu(ClO_4)_2$), surfactants (1μM sodium dodecylbenzenesulphonate (SDS), TWEEN-20, hexadecyl trimethyl ammonium bromide (CTAB)), some molecules with similar structures (1 μM ammonia, 1, 2-diaminoethane, aminoguanidine, and arginine), and some important biological molecules (1 μM glucose, phosphate and ATP, 1 μg/L DNA and BSA).

10. Extract of Raw Milk Samples: The liquid milk bought from local supermarket was pretreated according to the general procedure.[3] 5 g liquid milk containing streptomycin and 5 mL of 61 mM trichloroacetic acid were added into 35 mL of 4.9 M methanol solution. After 15 min sonication and 10 min shaking, the mixture was centrifuged at 10,000 rpm for 4 min, and the supernatant was filtrated. Then, the filtrate was concentrated to 10 mL and filtered through a 0.45 μm filter membrane for detection.

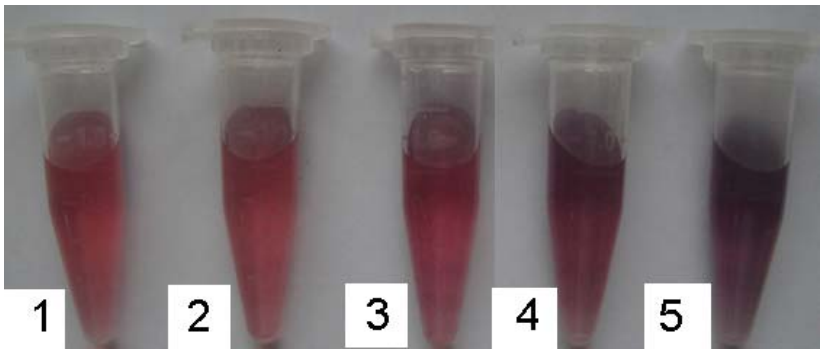

Fig. S8 Visual colorimetric change of the optimized Au NP probe: 1) without any addition; 2) with the addition of the extract from blank milk sample; 3) with the addition of the extract containing 1 ppm streptomycin (final concentration: 10 ppb); 4) with the addition of the extract containing 5 ppm streptomycin (final concentration: 50 ppb); 5) with the addition of the extract containing 10 ppm streptomycin (final concentration: 100 ppb).

11. REFERENCES

[1] G. Frens, *Nat. Phys.,* **1973**, 241, 20-22.

[2] S. Link, M. El-Sayed, *J. Phys. Chem. B* 1999, *103,* 8410-8426.

[3] Z. Wu, H. Zhao, Y. Xue, Q. Cao, J. Yang, Y. He, X. Li, Z. Yuan, *Biosens. Bioelectron.,* **2011**, 26, 2574-2578.