Supplemental Information

Mechanistic Study of the Synergistic Antibacterial Activity of Combined Silver Nanoparticles and Common Antibiotics

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Supplemental materials include:

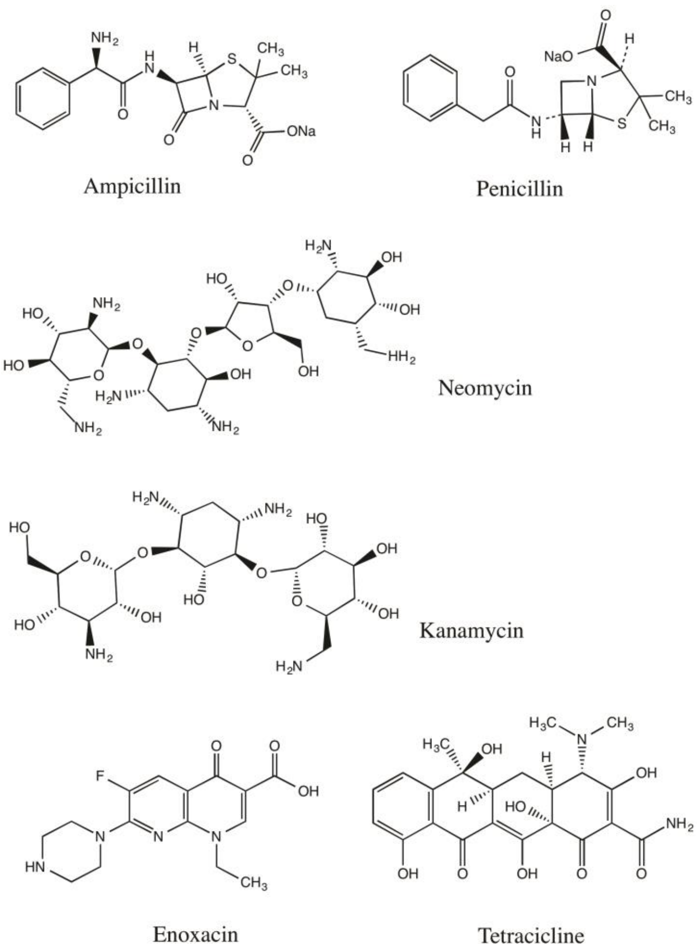

Figure SI 1. Structures of ampicillin (AMP), penicillin (PEN), neomycin (NEO), kanamycin (KAN), enoxacin (ENO) and tetracycline (TET).

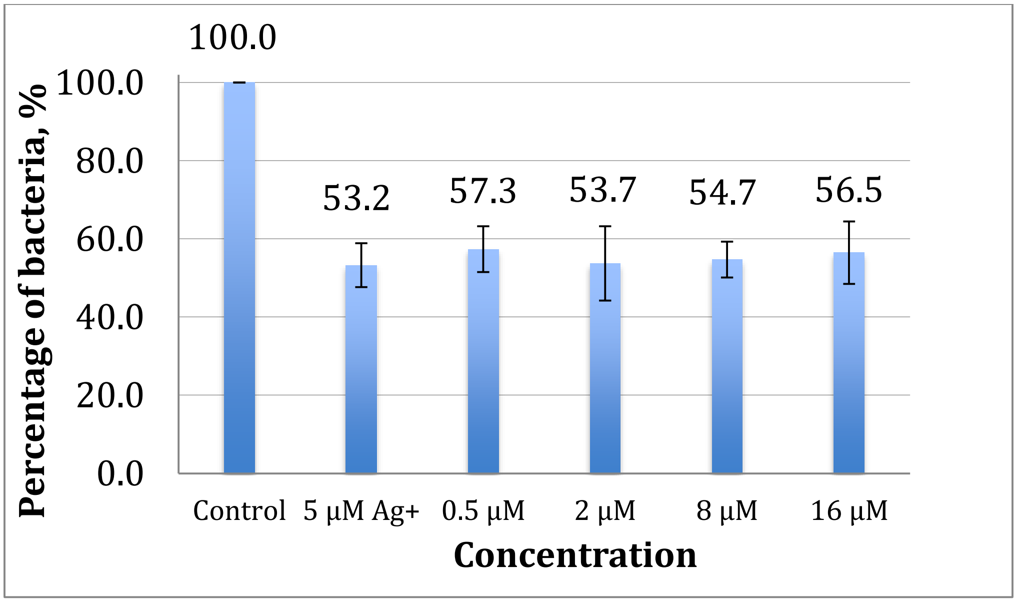

Figure SI 2. Inhibition of 5 μM Ag^+ combined with 0, 0.5, 2, 8, and 16 μM tetracycline against 1x10^5 CFU/mL *Salmonella* cells. Average counts were obtained from three repeats.

Additive antibacterial effect of Ag^+ and tetracycline against *Salmonella*

As an important control, it needs to be confirmed whether Ag^+ has synergistic effect with antibiotics. Tetracycline was chosen as a representative. 5 μM Ag^+ was combined with 0, 0.5, 2, 8, and 16 μM tetracycline to test their inhibition against 1x10^5 CFU/mL bacterial cells. All experimental conditions remain the same with AgNPs groups. As shown in figure SI 2, all groups inhibit 42.7% - 46.8% of bacterial growth with no significant difference, demonstrating that there is no synergistic effect between Ag^+ and tetracycline.

No evidence shows Ag^+ and tetracycline have interaction to form “complex” like that between AgNPs and tetracycline. Therefore, Ag^+ and tetracycline must carry out antibacterial activity separately even when they are combined together. This means there should be an additive antibacterial effect between these two antibiotics. However, since the *Salmonella* is resistant to tetracycline, the inhibition results observed in this test are actually caused by Ag^+.

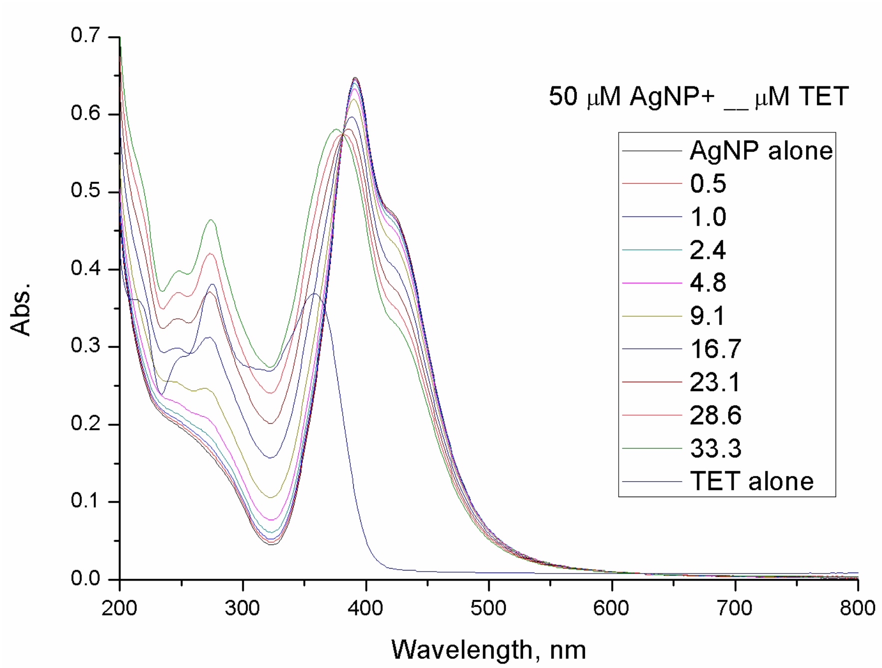

Figure SI 3. UV-vis absorption of titration of 100 μM tetracycline (TET) into AgNPs.

UV-vis titration of tetracycline into AgNPs solution

In the experiments of Ag binding to *Salmonella* cells and the Ag^+ release in the presence of bacterial cells, centrifugation is the key method to separate bacteria cells from AgNPs in solution or separate released Ag^+ from solutions. If AgNPs would aggregate, this method would not work. To confirm it, 100 μM tetracycline was titrated into 50 μM AgNPs solutions and characterized by UV-vis absorption. The concentration of tetracycline increased from 0 to 33 μM during the titration, but there is no sign of aggregation, as shown in figure SI 2. This proves that in this concentration range of tetracycline used, it does not cause AgNPs to aggregate.

Table SI 1. Choice of centrifuge speed to separate *Salmonella* cells from AgNPs solutions. AgNPs (100 µM) and 10^6-10^7 CFU/mL *Salmonella* solutions were used to test how centrifuge spin rate affects the settlement of the cells or the AgNPs. At 200 rpm, nearly 100% cells settle. Then different concentrations of AgNPs solutions were used to test the validity of the separation method of centrifuge at 2,000 rpm for 15 min (below table).

Centrifugation speed, rpm	100 µM AgNPs solution, % remaining in supernatant after centrifugation		10^6-10^7 CFU/mL *Salmonella* solution, % remaining in supernatant after centrifugation	
	Average	SD	Average	SD
0	100.00	0.00	100.00	0.00
500	99.63	2.40	97.64	2.50
1000	96.15	2.68	83.58	13.00
1500	94.09	2.64	30.27	15.34
2000	**88.72**	**1.28**	**5.76**	**5.46**
2500	80.64	1.71	3.63	4.18
3000	68.64	2.45	0.44	0.83
3500	60.78	2.26	\	\

	% remaining in supernatant after centrifugation at 2,000 rpm for 15 min	
	Average	SD
AgNPs 100µM	88.72	1.28
AgNPs 50µM	91.58	1.85
AgNPs 20µM	92.47	1.3
AgNPs 10µM	94.19	0.58
AgNPs 5µM	96.31	1.27
AgNPs 1µM *	**99.10 ***	**3.95 ***

Table SI 2. Ag^+ release concentrations and percentages from 1 μM and 50 μM AgNPs solutions in presence of various antibiotics (10 μM).

AgNPs concentrations	1 μM		50 μM	
	Ag^+, nM	Ag release, %	Ag^+, nM	Ag release, %
AgNPs	96.9 ± 20.1	9.7 ± 2.0	1046.1 ± 258.3	2.1 ± 0.5
AgNPs+ 10 μM AMP	103.2 ± 27.4	10.3 ± 2.7	1068.1 ± 344.1	2.1 ± 0.7
AgNPs+ 10 μM PEN	97.4 ± 43.6	9.7 ± 4.4	1145.3 ± 319.0	2.3 ± 0.6
AgNPs+ 10 μM ENO	134.1 ± 34.9	13.4 ± 3.5	1395.6 ± 307.1	2.8 ± 0.6
AgNPs+ 10 μM KAN	134.9 ± 36.3	13.5 ± 3.6	1485.3 ± 372.8	3.0 ± 0.7
AgNPs+ 10 μM NEO	154.2 ± 27.6	15.4 ± 2.8	1579.3 ± 341.6	3.2 ± 0.7
AgNPs+ 10 μM TET	144.8 ± 20.7	14.5 ± 2.1	1446.6 ± 281.5	2.9 ± 0.6

Effect of antibiotics on Ag^+ release

As shown in table SI 2, ampicillin and penicillin have no significant effect on the Ag^+ release from 1 μM or 50 μM AgNPs solutions. Enoxacin, kanamycin, neomycin and tetracycline enhance Ag^+ release by about 3-5% and 0.9-1.1% in cases of 1 μM and 50 μM AgNPs, respectively. Results both AgNPs concentrations confirm that the interaction between antibiotics and AgNPs (formation of complexes) facilitate the Ag^+ release from AgNPs.

When the concentration of AgNPs increases from 1 μM to 50 μM (50 times increase), the released Ag^+ concentrations don't increase by 50 times, but by 10 times. And the Ag^+ release percentage even decreased by about 75%, from 8% decreased to 2% in AgNPs only group. This demonstrates no linear relationship between Ag^+ release and AgNPs concentration. In other words, AgNPs concentration influences Ag^+ release but the whole equilibrium system of solution determines the final concentration of Ag^+.

Table SI 3. Ag^+ release concentrations and percentages from 1 μM and 50 μM AgNPs solutions in presence of *Salmonella* cells.

Concentrations	1 μM		50 μM	
	Ag^+, nM	Ag release, %	Ag^+, nM	Ag release, %
AgNPs	96.9 ± 20.1	9.7 ± 2.0	1046.1 ± 258.3	2.1 ± 0.5
AgNPs + Cell	120.8 ± 34.1	12.1 ± 3.4	1181.3 ± 201.3	2.4 ± 0.4
AgNPs +10 μM PEN	97.4 ± 43.6	9.7 ± 4.4	1145.3 ± 319.0	2.3 ± 0.6
AgNPs + 10 μM PEN + Cell	122.8 ± 25.7	12.3 ± 2.6	1274.4 ± 305.4	2.5 ± 0.6
AgNPs + 10μM TET	144.8 ± 20.7	14.5 ± 2.1	1446.6 ± 281.5	2.9 ± 0.6
AgNPs + 10 μM TET + Cell	182.4 ± 25.8	18.2 ± 2.6	1627.7 ± 203.3	3.3 ± 0.4

Effect of *Salmonella* cells on Ag^+ release

The presence of *Salmonella* further enhances Ag^+ release in all experimental groups regardless of the AgNPs concentrations, as shown in table SI 3. The Ag^+ ion release from AgNPs depends on the coating agents, size and concentration of AgNPs as well as other environmental factors, including temperature, pH values, dissolved dioxygen and protons [1-3]. In current experimental conditions, antibiotic molecules that have interaction with AgNPs enhance the Ag^+ ion release from AgNPs. Since AgNPs surface absorbs and is covered with Ag^+ ions, the interaction between antibiotics and AgNPs facilitates the release of Ag^+ ion. Especially, the competition between antibiotics and citrate causes the leaving of citrate ions, which helps the leaving of Ag^+ ions from AgNPs by forming citrate complex with Ag^+ [4]. On the other hand, the presence of antibiotics will bring an environment of more dissolved dioxygen and protons, promoting conversion from Ag(0) to Ag(I) [5, 6]. For the same reason, the complex biological system of bacterial cells will promote conversion of AgNPs to Ag(I) and trigger enhanced Ag^+ release.

Table SI 4. Estimated number of released Ag^+ from every surface layer of AgNPs.

Layer of a single AgNP (outside to inside)	Radius r, nm	Surface area, S= $4\pi r^2$, nm^2	Estimated Number, N = S/S_{Ag}, × 10^4	Total Ag^+ upon complete release, × 10^4	Total Ag^+ release of 30 AgNPs, × 10^5	Total Ag^+ release of 37 AgNPs, × 10^5	Total Ag^+ release of 73 AgNPs, × 10^5
1	14.9	2789.9	3.0	3.0	9.0	11.1	21.9
2	14.6	2662.5	2.9	5.9	17.6	21.7	42.8
3	14.2	2538.2	2.7	8.6	25.8	31.8	62.8
4	13.9	2416.8	2.6	11.2	33.6	41.4	81.7
5	13.5	2298.4	2.5	13.7	41.0	50.6	99.8
6	13.2	2182.9	2.3	16.0	48.1	59.3	116.9
7	12.8	2070.5	2.2	18.2	54.7	67.5	133.2
8	12.5	1961.0	2.1	20.4	61.1	75.3	148.6
9	12.1	1854.5	2.0	22.4	67.1	82.7	163.2
10	11.8	1750.9	1.9	24.2	72.7	89.7	176.9
11	11.5	1650.4	1.8	26.0	78.0	96.2	189.9
12	11.1	1552.8	1.7	27.7	83.0	102.4	202.1

Notes: Average diameter of 29.8 nm is used for spherical AgNPs, giving an original radius of 14.9 nm. Van der Waals radius of Ag atom is known to be 172 pm, yielding an area of AgNPs cross-section of $S_{Ag} = \pi r^2 = 0.09294\ nm^2$.

Estimation of Ag^+ release from each AgNP

The rough number of Ag atoms on the surface of every single AgNP is estimated by the surface area of AgNPs dividing by the cross section area of a single Ag atom, as listed in table SI 4. Assuming Ag^+ ions are released from the surface of AgNPs layer by layer, we can estimate the Ag^+ release capability of different layer of every single AgNP.

In the case of 1 μM AgNPs interacting with 1×10^7 CFU/mL *Salmonella*, 30 and 37 AgNPs out of 73 AgNPs have interaction or bind to every *Salmonella* cell in absence and presence of tetracycline, respectively. And released Ag^+ concentrations are 121 and 182 nM, indicating Ag^+ ions to *Salmonella* cell ratios are $7.28 \times 10^6 : 1$ and $10.96 \times 10^6 : 1$ in absence and presence of tetracycline, respectively. There is an increase of 3.68×10^6 Ag^+ release triggered by a single *Salmonella* cell. Compared with total Ag^+ release of 37 AgNPs listed in table SI 4, combination of tetracycline and *Salmonella* cells facilitate the release of about 3 layers of Ag on the surface of AgNPs.

References

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