

Particle

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Supporting Information

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Single-Cell Investigations of Silver Nanoparticle–Bacteria Interactions

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S1. Supporting information 1

TEM image of silver nanoparticles (AgNPs)

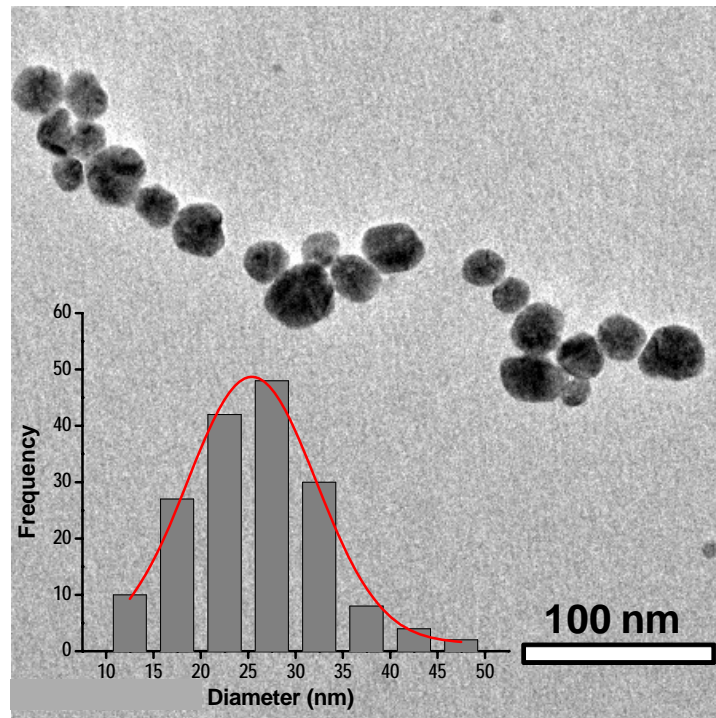


Figure S1. TEM image of silver nanoparticles (AgNPs). Inset: Size distribution of AgNPs plotted from multiple images. Synthesized NPs were of the size 25 ± 8.5 nm. This polydispersity is common in citrate reduced AgNPs.

S2. Supporting information 2

Hyperspectral image of AgNPs and corresponding scattering spectra

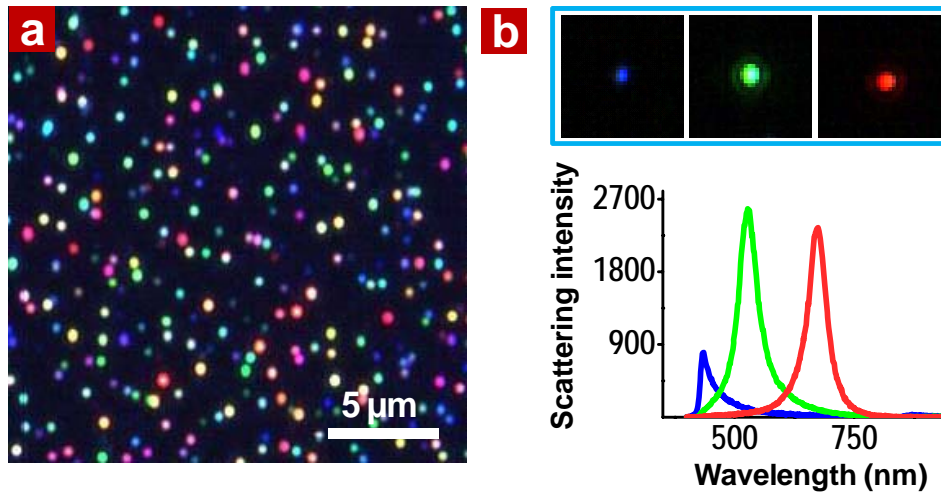


Figure S2. a) Large area hyperspectral image of AgNPs showing the polydisperse nature of the particles, b) enlarged images of single AgNPs of different sizes are shown with their respective scattering spectra.

S3. Supporting information 3

Hyperspectral image and scattering spectrum of untreated bacteria

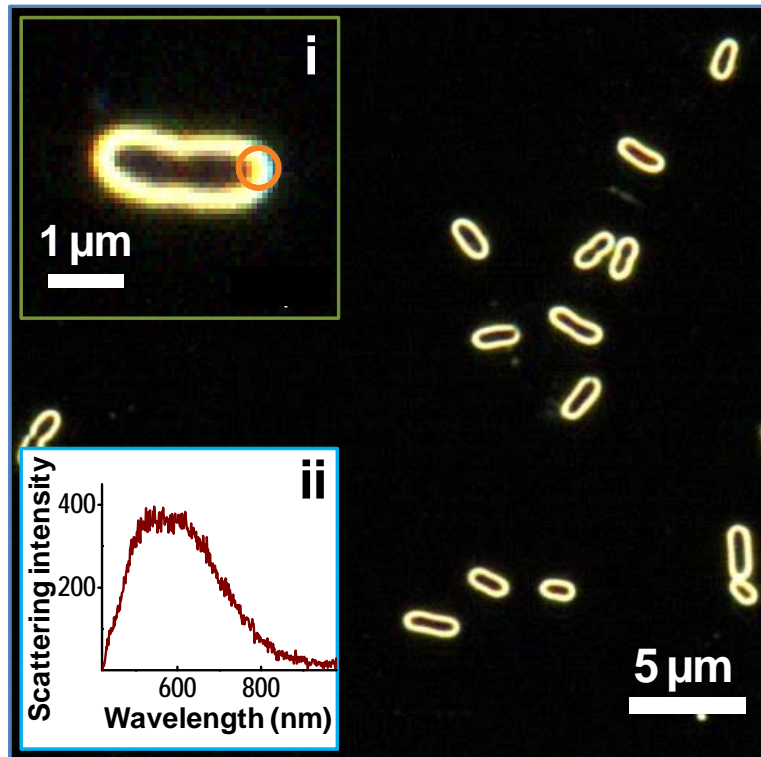


Figure S3. Large area hyperspectral image of untreated bacteria (control). (i) Enlarged image of a single bacterium and (ii) scattering spectrum of same bacterium taken from the encircled region in (i). Note the reduced intensity in comparison to the scattering spectrum from AgNPs (Figure S2). Images have been enhanced digitally.

S4. Supporting information 4

Time dependent study of AgNPs treated bacteria using HSI

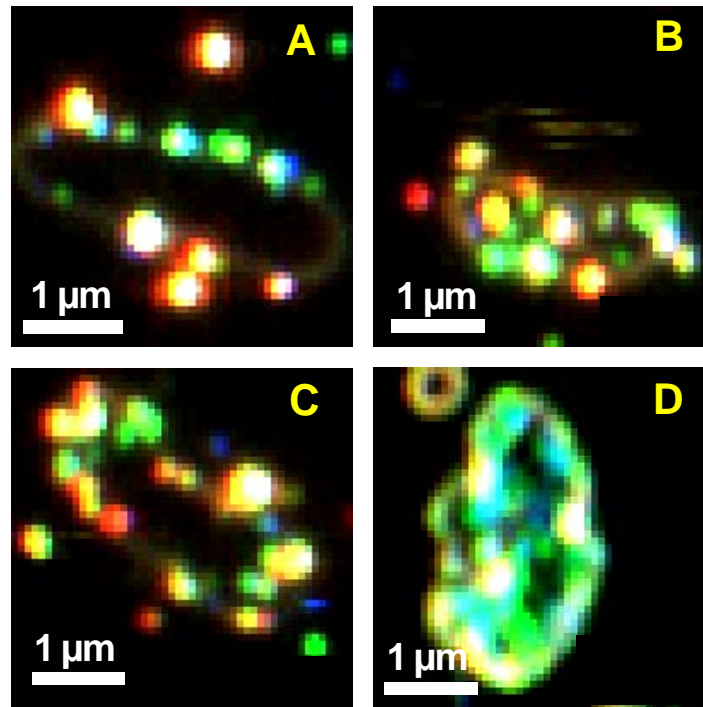


Figure S4. Hyperspectral images of AgNPs treated bacteria for various incubation periods A) 5 min, B) 10 min, C) 30 min and D) 60 min. Increase in the population of NPs attached to the bacterium can be seen with the increase in incubation time. Distortion in the morphology of the bacterium was seen after 60 minutes. The bacterial cell membrane is seen upon closer examination.

S5. Supporting information 5

Time dependent Raman measurements

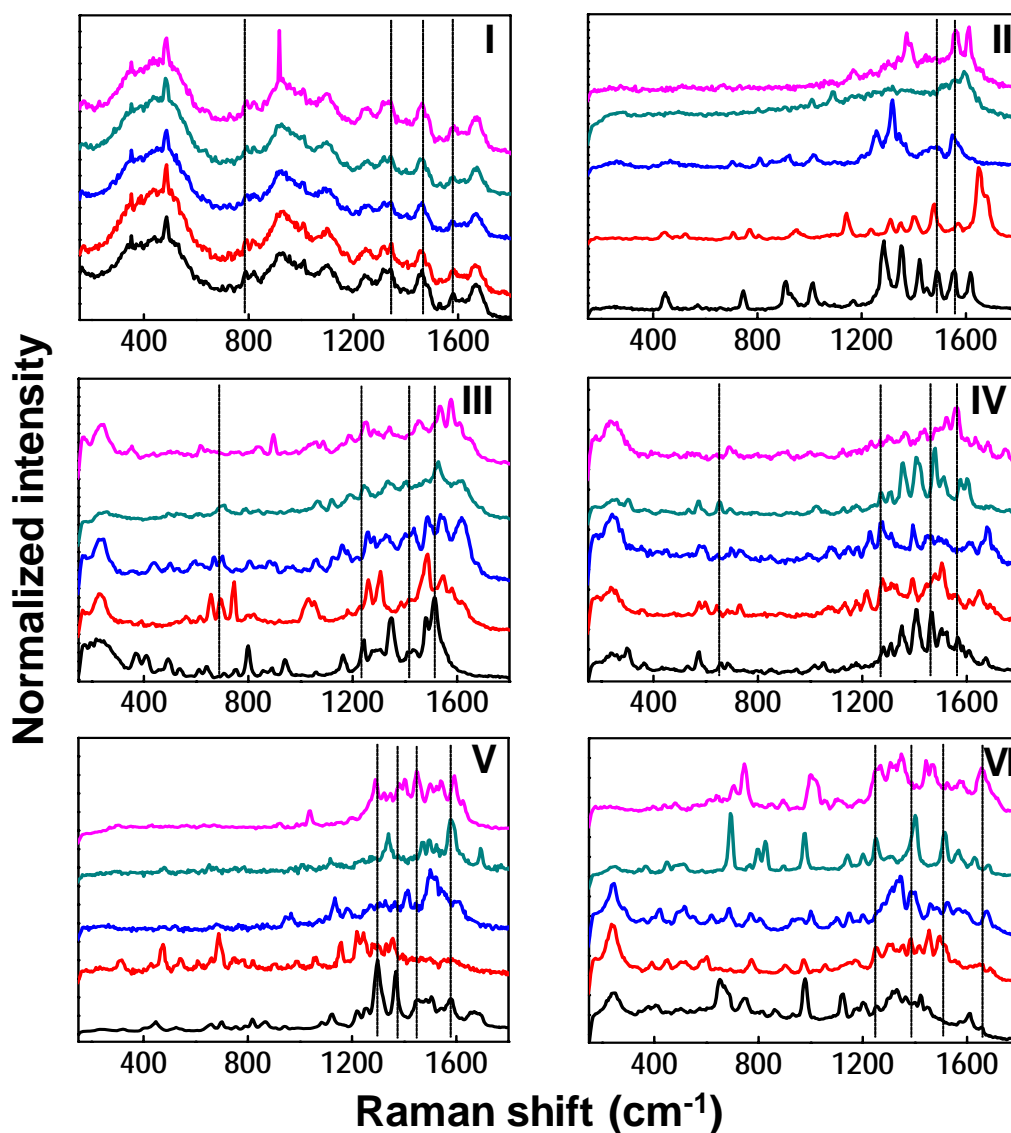


Figure S5. Comparison of the Raman spectra of untreated bacteria (I) and bacteria treated with AgNPs for increasing incubation times: 5, 10, 20, 30 and 60 minutes (II, III, IV, V and VI), respectively. To prove the reproducibility of Raman spectra, spectra from five different bacteria at each time point were measured and compared. These are stacked in each set. Reproducible features of DNA are marked with lines. It may be noted that reproducibility does not imply exact peak shape. Assignments of the peaks were done using previous reports on Raman spectroscopy of bacteria (Table S6).^[31-39]

S6. Supporting information 6

Table S6. Assignments of Raman peaks of untreated and AgNPs-treated *E. coli* cells for various time intervals: 5, 10, 20, 30 and 60 minutes. Assignments were done by matching with reported frequencies within ± 7 cm^{-1} . Note: bk, dAMP, dGMP, dCMP and dUMP refer to backbone, deoxyadenosine monophosphate, deoxyguanosine monophosphate, deoxycytosine monophosphate and deoxyuridine monophosphate, respectively. The assignments are based on various reports.^[31-39]

S. No.	Peaks (cm^{-1})	Assignments	S. No.	Peaks (cm^{-1})	Assignments
1.	657	Guanine, Tyrosine ^[36]	24.	1341	Adenine, Guanine of DNA/RNA, CH def. of protein ^[31]
2.	677, 685	dGMP ^[32]	25.	1342	Tryptophan ^[32]
3.	726	Adenine ^[39]	26.	1347	Adenosine and Guanosine ^[37]
4.	769	Tryptophan ^[33]	27.	1353	Tryptophan ^[37]
5.	789	dCMP ^[32]	28.	1385	Adenine, Guanine ^[37]
6.	930	DNA bk ^[39]	29.	1392, 1393, 1396	Uridine ^[38]
7.	979	Membrane phospholipids ^[35]	30.	1444	Guanine, Adenine, CH def. of DNA/RNA, CH def. of proteins, lipids, carbohydrates ^[31]
8.	1002	Phenylalanine ^[31]	31.	1462, 1463	Guanine, Adenine, CH def. of DNA/RNA, CH def. of proteins, lipids, carbohydrates ^[31]
9.	1011, 1012	Tryptophan ^[32]	32.	1471	dUMP ^[32]
10.	1037	Phenylalanine protein ^[31]	33.	1480, 1481, 1482	dAMP ^[32]
11.	1059	Lipid layer fluidity ^[35]	34.	1503	Adenosine ^[38]
12.	1069	C-N str. of proteins, chain C-C str. of lipids ^[31]	35.	1522, 1525	Cytidine ^[34]
13.	1090	DNA:O-P-O ⁻ ^[39]	36.	1529	dCMP ^[32]
14.	1128, 1132	Protein/lipids/carbohydrates ^[31]	37.	1537	Cytidine ^[37]
15.	1178	Tyrosine ^[33]	38.	1556	Tryptophan ^[32]

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16.	1185	Tyrosine ^[32]	39.	1570, 1572	Guanine+Adenine ^[33]	
17.	1230, 1232	Uridine ^[38]	40.	1581	dAMP ^[32]	
18.	1243, 1244	Thymidine ^[38]	41.	1592	Adenine ring stretching ^[36]	
19.	1251, 1252	Cytidine ^[38]	42.	1602	Phenylalanine ^[32]	
20.	1275, 1278	Thymidine ^[38]	43.	1610	Phenylalanine, Tyrosine ^[31]	
21.	1289	dCMP ^[32]	44.	1613, 1616	Tryptophan+Tyrosine ^[33]	
22.	1314, 1315	Guanine of DNA/RNA, CH def. of protein ^[31]	45.	1657	dCMP ^[32]	
23.	1328	Adenine, Tyrosine ^[34]	Guanine,	46.	1683	dGMP ^[32]

S7. Supporting information 7

Optical images of *E. coli* treated with AgNPs for various time intervals

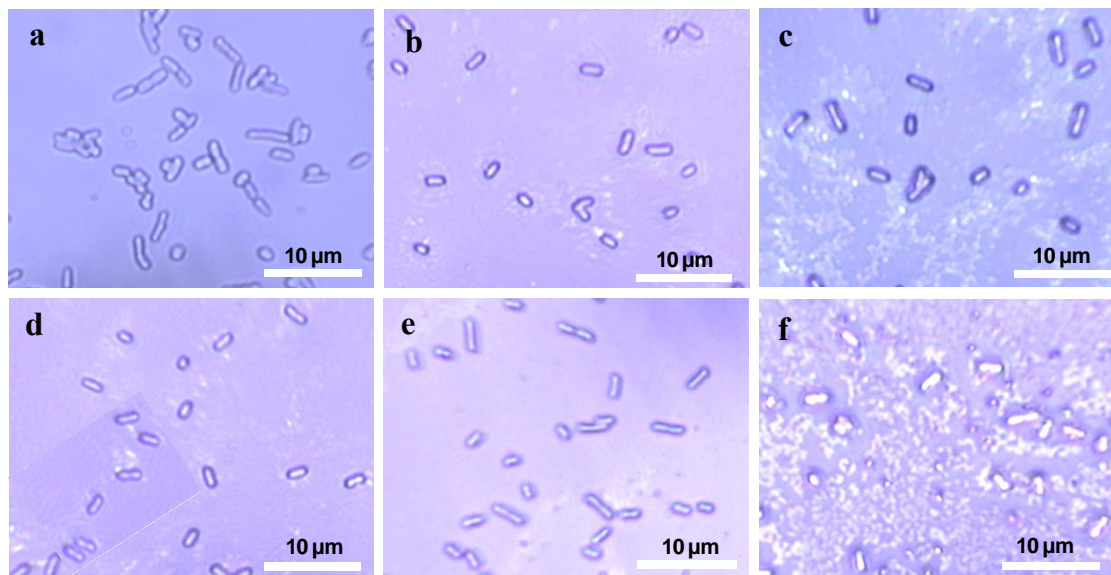


Figure S7. Optical images of untreated bacteria and bacteria treated with AgNPs. Control bacteria (a), and bacteria treated with silver NPs for various incubation times: 5, 10, 20, 30 and 60 minutes (b, c, d, e and f, respectively). Visible change in the bacterial morphology was observed upon longer incubation (f).

S8. Supporting information 8

Table S8. Raman peak assignments of plasmid DNA isolated from control *E. coli* treated with AgNPs. Assignments were done considering an error of $\pm 6 \text{ cm}^{-1}$. Note: **d**–vibration localized in the deoxyribose moiety, **dA**, **dT**, **dG**, **dC**–deoxynucleosides A, T, G, C, **bk**-DNA backbone, **δ** – deformation vibration.^[45] The assignments are based on various literature reports.^[32,36,38,43-45]

S. No.	Peaks (cm^{-1})	Assignments	S. No.	Peaks (cm^{-1})	Assignments
1.	1670	dT (C=O str.) ^[43,44]	15.	1331	Guanosine ^[38]
2.	1605	dC, dG, dA ^[44]	16.	1315	dG ^[44]
3.	1591	Adenine ring stretching ^[36]	17.	1300	Cytidine ^[38]
4.	1580	Adenosine ^[44]	18.	1282	Cytosine ^[32]
5.	1573	Guanosine ^[38]	19.	1260	dA, dC ^[45]
6.	1524	dC ^[44]	20.	1243	dT, dC ^[44]
7.	1512	dA ^[44]	21.	1172	dG ^[44]
8.	1478	Adenosine ^[38]	22.	1151	dT ^[44]
9.	1462	d(5'-CH ₂ δ) ^[44]	23.	1098	bk (PO ₂ ⁻ str.) ^[43,44]
10.	1440	d(CH ₂ δ) ^[44]	24.	1085	γ (PO ₂ ⁻) ^[45]
11.	1410	Thymidine ^[38]	25.	1001	d ^[44]
12.	1377	dT ^[43]	26.	976	d ^[44]
13.	1366	Guanosine ^[38]	27.	923	d ^[44]
14.	1339	dA, dG ^[44]	28.	684	dG ^[43,44]

S9. Supporting information 9

Time dependent Raman analysis of *E. coli* with Ag^+ ions

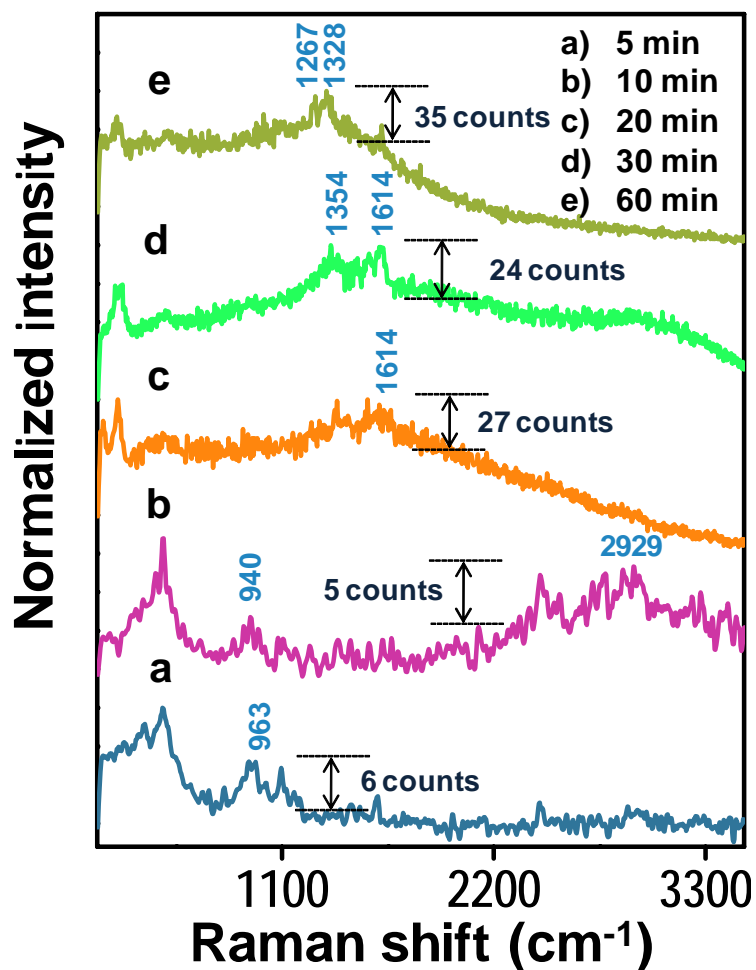


Figure S9. Raman spectra of *E. coli* treated with Ag^+ ions for various time intervals: 5, 10, 20, 30 and 60 minutes. There was no enhancement of peaks in the Raman spectra. Reproducible features are marked on the traces and their assignments are listed in Table S10.^[27,31-35,37]

S10. Supporting information 10

Table S10. Assignments of Raman peaks of *E. coli* treated with Ag⁺ ions for various incubation periods (5, 10, 20, 30 and 60 minutes).^[27,31-35,37]

S. No	Peaks (cm ⁻¹)	Assignments
1.	940	α -helix of protein, carbohydrate ^[31]
2.	963	Membrane phospholipids ^[35]
3.	1354, 1555, 1614	Tryptophan ^[37,32,33]
4.	1267	C-O-C modes of pyranose rings ^[27]
5.	1328	Tyrosine ^[34]
6.	2929	C-H stretching region ^[35]

S11. Supporting information 11

Time dependent HSI analysis of *E. coli* treated with Ag^+ ions

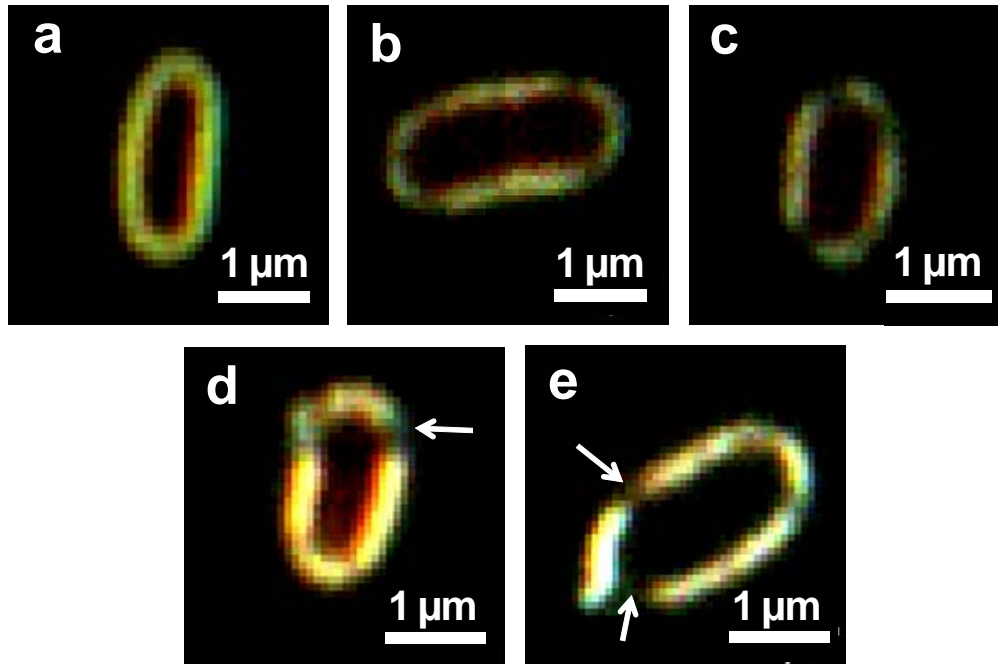


Figure S11. Images obtained from hyperspectral analysis of Ag^+ ion treated *E. coli* for various incubation periods a) 5 min, b) 10 min, c) 20 min, d) 30 min and e) 60 min. For incubation time more than 30 minutes, lysis of the cell membrane of the bacterium was seen as indicated by arrow.

S12. Supporting information 12

ITC measurement of the interaction of AgNPs with bacteria

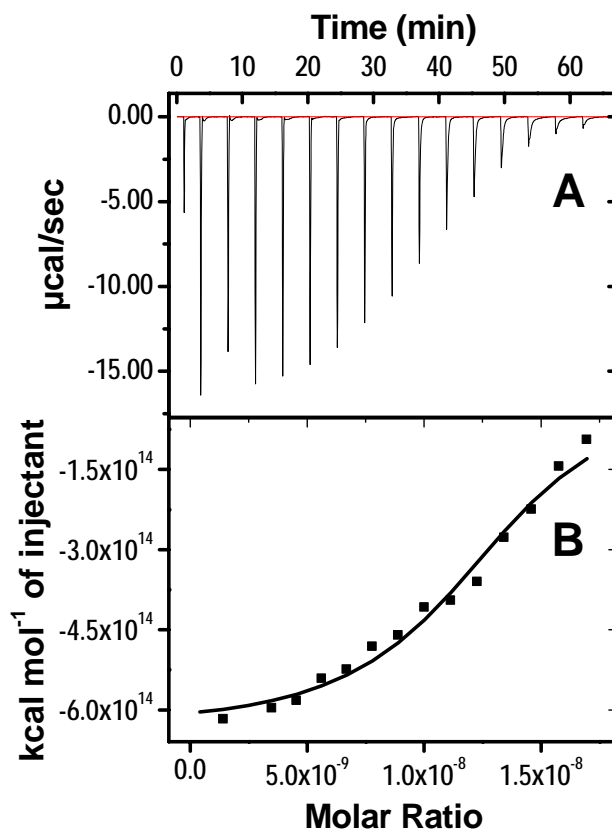


Figure S12. (A) ITC responses for the titration of *E. coli* with the AgNPs. (B) Integrated calorimetric response plotted against the molar ratio of *E. coli*/AgNPs (concentrations – 1.62×10^{-14} M/ 1.2×10^{-9} M). Volume of $2.5 \mu\text{L}$ was injected for every injections with the time gap of 250 seconds.