Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information

It's Not All About the Ion: Support for Particle-Specific Contributions to Silver Nanoparticle Antimicrobial Activity

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Total Ag atom calculations

Ag salt control

Under the alternate assumption – that the reported concentration was of total Ag salt rather than Ag atom – the molecular weight would change from 107.87 g/mol (corresponding to Ag) to that of the Ag salt (e.g., 169.87 g/mol for $AgNO_3$, 166.91 g/mol for $AgC_2H_3O_2$, 311.80 g/mol for Ag_2SO_4). The molar ratio would also need to be incorporated here. For a reported concentration of $0.4\,\frac{\mu g}{mL}$ $AgNO_3$:

$$0.4\,\frac{\mu g}{mL} AgNO_3 * \frac{1\ g}{10^6\ \mu g} * \frac{1\ mol}{169.87\ g} * \frac{1\ mol\ Ag}{1\ mol\ AgNO_3} = 2.35\ x\ 10^{-9} \frac{mol\ Ag(I)}{mL} as\ AgNO_3,$$

which is not significantly different from $3.71x10^{-9}\frac{mol\ Ag(I)}{mL} as\ AgNO_3$ (assuming the reported concentration is of total Ag atom).

AgNP

The alternate assumption – that the reported concentration was of total AgNP rather than Ag atom – requires including the mass of the ligand, which depends on the number of ligands appended to the particle. Since the majority of studies do not include this information, the mass of the ligand was ignored for the purposes of this example calculation. For a 25 nm particle, the number of total Ag atoms and thus the weight of the AgNP is:

$$\frac{4.85x10^5\ atoms\ Ag}{AgNP}(for\ a\ 25\ nm\ AgNP) * \left(\frac{107.87\ g}{1\ atom\ Ag}\right)$$
$$= \left(\frac{5.19x10^7 g\ Ag}{AgNP}\right) * \left(\frac{6.02x10^{23} AgNPs}{1\ mol\ AgNPs}\right) = \frac{3.13x10^{31} g\ Ag}{1\ mol\ AgNPs}$$

For a reported concentration of $0.4\,\frac{\mu g}{mL}\,AgNPs$:

$$0.4\frac{\mu g}{mL}\,AgNPs * \left(\frac{1\ g}{10^6\ \mu g}\right) * \left(\frac{1\ mol\ AgNP}{3.13x10^{31} g\ Ag}\right) = 1.28x10^{-36}\ mol\frac{AgNPs}{mL}.$$

The associated extent of necessary AgNP oxidation is unrealistic (10^{29}%) and resulted in rejection of every study for having an overestimated ion control.

Surface Ag atom calculations

General considerations for Ag

Ag has face-centered cubic (FCC) lattice structure:

$$Unit\ cell = 6 * \frac{1}{2} + 8 * \frac{1}{8} = 4\ \frac{Ag\ atoms}{unit\ cell}$$
$$Unit\ cell = 4 * \frac{1}{8} + 1 * \frac{1}{2} = 1\ \frac{Ag\ atom}{surface\ unit\ cell}$$

$$Ag\ lattice\ constant\ (a_{lat}) = 0.408\ nm$$
$$Unit\ cell\ volume\ (nm^3) = {a_{lat}}^3$$
$$Bond\ length\ (BL) = 2r = \frac{\sqrt{2}a_{lat}}{2} = \frac{a_{lat}}{\sqrt{2}}$$

$$SA_{(111)} = \frac{\sqrt{3}BL^2}{2}$$
$$SA_{(110)} = BL * a_{lat}$$
$$SA_{(100)} = BL^2$$

$$Unit\ cell\ surface\ area\ (nm^2) = (\%(111)) * (SA_{(111)}) + (\%(110)) * (SA_{(110)}) + (\%(100)) * (SA_{(100)})$$

$$Volume\ (nm^3)\ x \left(\frac{1\ unit\ cell}{V\ (nm^3)}\right) x \left(\frac{4\ Ag\ atoms}{unit\ cell}\right) = total\ number\ of\ Ag\ atoms$$
$$Surface\ area\ (nm^2)\ x \left(\frac{1\ unit\ cell}{SA\ (nm^2)}\right) x \left(\frac{1\ Ag\ atom}{unit\ cell}\right) = number\ of\ surface\ Ag\ atoms$$

Sphere approximated as a cuboctahedron

$$Particle\ radius\ (r) = 25\ nm$$
$$V_{sphere} = \frac{4}{3}\pi r^3 = 65{,}450\ nm^3$$
$$SA_{sphere} = 4\pi r^2 = 7{,}854\ nm^2$$

A cuboctahedron has 8 (111) faces as equilateral triangles and 6 (100) faces as squares.[1] The relative percentage of each surface facet was determined using the edge length (E) as it defines the size of the 8 triangles and 6 squares:

$$A_{8\ triangles} = 8 * \frac{b * h}{2} = 8\frac{E * E\frac{\sqrt{3}}{2}}{2} = 2 * \sqrt{3} * E^2$$
$$A_{6\ squares} = 6E^2$$
$$A_{total} = A_{8\ triangles} + A_{6\ squares}$$
$$\%\ (111) = \frac{A_{8\ triangles}}{A_{total}} = \frac{2 * \sqrt{3} * E^2}{2 * \sqrt{3} * E^2 + 6E^2} = 0.366$$
$$\%\ (100) = \frac{A_{6\ squares}}{A_{total}} = \frac{6E^2}{2 * \sqrt{3} * E^2 + 6E^2} = 0.634$$

$$Unit\ cell\ surface\ area\ (nm^2) = (0.366) * (SA_{(111)}) + (0) * (SA_{(110)}) + (0.634) * (SA_{(100)}) = 0.079$$
$$Total\ number\ of\ Ag\ atoms = 3,854{,}678\ Ag\ atoms$$
$$Number\ of\ Ag\ surface\ atoms = 99,228\ Ag\ surface\ atoms$$

$$\%\ Ag\ surface\ atoms = \%\ ionization = \frac{Ag\ surface\ atoms}{total\ Ag\ atoms} x\ 100 = 2.57\%$$

Nanorod/wire approximated as a cylinder with half spheres on each end

$$Length\ (l) = 6755\ nm$$
$$Width\ (w)/Diameter(d) = 82\ nm$$
$$V_{total} = V_{sphere} + V_{cylinder} = \frac{4}{3}\pi r^3 + \pi r^2 h = 35{,}528{,}924\ nm^3$$
$$SA_{total} = SA_{sphere} + SA_{cylinder} = 4\pi r^2 + 2\pi r h = 1{,}740{,}159\ nm^2$$

A nanorod/wire has 2 (111) faces as the half spheres while the side surface is a (100) face.[2] The relative percentage of each surface facet was determined using the edge length (E) as it defines the size of the 8 triangles and 6 squares:
$$A_{sphere} = 4\pi r^2$$
$$A_{cylinder} = 2\pi r h$$
$$A_{total} = A_{sphere} + A_{cylinder}$$
$$\%\ (111) = \frac{A_{sphere}}{A_{total}} = \frac{4\pi r^2}{4\pi r^2 + 2\pi r h} = 0.0121$$
$$\%\ (100) = \frac{A_{cylinder}}{A_{total}} = \frac{2\pi r h}{4\pi r^2 + 2\pi r h} = 0.988$$

$$Unit\ cell\ surface\ area\ (nm^2)$$
$$= (0.0121) * (SA_{(111)}) + (0) * (SA_{(110)}) + (0.988) * (SA_{(100)}) = 0.083$$
$$Total\ number\ of\ Ag\ atoms = 2,092{,}481{,}147\ Ag\ atoms$$
$$Number\ of\ Ag\ surface\ atoms = 20{,}941{,}396\ Ag\ surface\ atoms$$

$$\%\ Ag\ surface\ atoms = \%\ ionization = \frac{Ag\ surface\ atoms}{total\ Ag\ atoms} x\ 100 = 1.00\%$$

Table S1. Compilation of qualitative and empirical data reported in the final subset of studies used for the pivot table analysis

Refs	Species or organism group	Exposure medium	AgNP size (nm)[a]	AgNP shape	Surface ligand (zeta potential mV)	Ligand control present	AgNP synthesis method	Purification procedure	AgNP conc. characterization method	Aggregation monitoring method (time)	Additional characterization	Assay	Endpoint	Conclusion drawn
3	*E. coli* nonmutated Strain BW25113, biosensor *E. coli* MC1061 (pcueR/pcopAlux) and MC106 (pSLlux)	LB with 5% FBS and some antibiotics	9.1 ± 4.2 19.1 ± 6.0 43.5 ± 12	sphere	citrate (-26.3 ± 2.6)	yes – not toxic	purchased from Nanocomposix	--	GF-AAS	DLS (duration)	--	growth, biosensor, gene deletion, AFM/TEM	IC_{50}, bioavailable Ag, gene regulation, AgNP-cell interaction	particle-only, combined ion-particle
			17.9 ± 7.0	sphere	PVP (-10.7 ± 1.8)	yes – not toxic	synthesized using $AgNO_3$ and $NaBH_4$	ultrafiltration system (Spectrum Laboratories, Inc.) equipped with a 10 kDa polyethersulfone membrane (MidiKros Hollow Fiber Module (P-X3-010E-300-02N))						
			23.3 ± 15	sphere	BPEI (+33.3 ± 1.5)	yes – not toxic	synthesized using $AgNO_3$ and UV irradiation							
4	*E. coli* K12 ATCC 25404	$NaHCO_3$ buffer	35.4 ± 5.1[b]	sphere	amorphous carbon (-27 ± 1.54 anaerobic, -30 ± 0.42 aerobic)	--	purchased from Novacentrix Corporation	washed 5 times with 1% HNO_3 and 5 times with deoxygenated (N_2-purged) water inside an anaerobic chamber, filtration through a cellulose membrane (MWCO 10 kDa) using an Amicon stir cell (Millipore, MA) pressurized with nitrogen	ICP-OES/MS	--	--	plate counting, dose-reponse, MLC assay	viable cell number (CFU), log reduction, MLC	ion-only, combined ion-particle
5	*E. coli* K12 ATCC 25404	$NaHCO_3$ minimal media	2.8 ± 0.47 4.7 ± 0.2 10.5 ± 0.59	sphere	PEG (-16.1 ± 1.2)	--	synthesized by reaction of tetrachloroauric acid or $AgC_2H_3O_2$ with oleylamine in refluxing toluene, hexane, or 1,2-dichloro-benzene	--	--	--	--	growth inhibition	EC_{50}, % live dead	ion-only
			17.5 ± 2.9 51.4 ±18.7 71.5 ±20.3	sphere	PVP (-27.5 ± 1.4)	--	commercially available from NanoAmor	--			--			
6	*E. coli* K12 MG 1655	modified LB lacking NaCl	142 ± 20	sphere	--	--	synthesized in the vapor phase	--	--	--	SEM and XRD to characterize NP composition, BET surface area	timed release Ag toxicity, growth curve, viable plate counts, TEM, RT-PCR	viable cell number (CFU), AgNP-cell interaction, fold change	combined ion-particle
7	*E. coli* K12 MG 1655	modified LB lacking NaCl	142 ± 20	sphere	--	--	synthesized in the vapor phase	--	--	--	SEM/EDX for composition	plate counts, growth curve, gene expression	viable cell number (CFU), fold change	combined ion-particle

												microarray		
8	*S. aureus* ATCC 6538, *E. coli* ATCC 10536	ISB, TSB, PBS Dulbecco's and HEPES, CAPS	7 ± 4	sphere	glutathione	--	mixed $AgNO_3$, sodium citrate, and $NaBH_4$.	Centrifugation and pellet precipitation	ICP-OES	UV-vis, DLS (duration)	Mie's effective refractive index for subsitution of the citrate capping layer, TGA, SEM EDS for composition, XRPD and FT-IR for capping agents	standard broth macro-dilution method, plate counting	ME, MIC	particle-only
9	*T. pseudonana* CCMP 1335, *Synechococcus* sp. PCC 6911	artificial seawater and freshwater	21.6	sphere	maltose (-38)	--	D-maltose monohydrate and $AgNO_3$ added to $NaBH_4$	--	--	DLS (duration)	CPS for size	dose response	% growth inhibition	combined ion-particle
			39		maltose (-49)		modified Tollens process - chemical reduction of $[Ag(NH_3)_2]+$ by sugars (mix of $AgNO_3$, NH_4OH, and NaOH - vary the latter two to control size	--						
			97.4		maltose (-48)			--						
10	*N. europaea* ATCC 19718	AOB medium	25.2 ± 9.3	sphere	citrate (-23 ± 1.1)	yes – not toxic	$AgNO_3$ reduced with citrate	--	ICP-MS	TEM, DLS	--	micro BCA protein assay, live/dead, TEM, gene expression	% reduction in nitrite production, membrane integrity, AgNP-cell interaction, fold change	ion-only
			27 ± 6.5	sphere	GA (-21 ± 1.3)	yes – not toxic	$AgNO_3$ reduced with GA	diluted and centrifuged three times before resuspending in water						
			21 ± 17	sphere	PVP (-11.6 ± 1.8)	yes – not toxic	purchased from Nanostructured and Amorphous Materials, Inc.	--						
11	PolySeed *(Bacillus* sp.)	BOD5 test medium	58 ± 11	sphere	hydrogen (-22)	yes – not toxic	Ag_2O heated and then bubbled with H_2	system from Spectrum Laboratories, Inc. ultrafiltration membrane 10 kDa polyethersulfone (MidiKros Hollow Fiber Module (P-X3-010E-300-02N))	AAS	DLS	XPS, NMR, and conductivity (Orion 013010MD conductivity cell) for purity	five day oxygen consumption test; live/dead Baclight, TEM	oxygen consumption, % live bacteria, AgNP-cell interaction	particle-only
			19 ± 5		citrate (-38)	yes – not toxic	$AgNO_3$ reduced with citrate							
			12[b]		PVP (-10)	yes – not toxic	$AgNO_3$ added dropwise to an $NaBH_4$ and PVP solution							
			10 ± 4		BPEI (+40)	yes – not toxic	BPEI and $AgNO_3$ dissolved in HEPES separately, mixed together, and exposed to UV light							
12	*E. coli* ATCC 25922	PBS	20.3 ± 1.9 53.1 ± 4.1	sphere	phosphate monolayer	--	bought from Nanocomposix	extensive washing	ICP-MS	TEM (duration)	DLS for HDD, UV-vis for size	plating assay	MBC	ion-only

			112.6 ± 7.8				(synthesized using aqueous reduction from Ag salts in phosphate buffer)							
13	*V. fischeri* DSMZ	2% NaCl	20	sphere	-11	--	purchased from PlasmaChem GmbH	--	ICP-MS	DLS	SEM, high-resolution (HR)-SEM, FTIR	standard inhibition of bioluminescence	% luminescence	combined ion-particle
			200		-12.65	--								
			23		-10.85	--	synthesized by *Ocimum sanctum* plant leaf extract							
			27		-13.5	--	synthesized by *Azadirachta indica* plant leaf extract							
14	*E. coli* AB1157 (pSLlux), *E. coli* JI130 (pSLlux), *E. coli* JI131 (pSLlux), *E. coli* AS393 (pSLlux), *E. coli* JI132 (pSLlux), *E. coli* AS391 (pSLlux), *E. coli* K12::lux, *E. coli* MC1061 (pDNlux), *E. coli* 12::soxRSsodAlux, *E. coli* MC1061 (pSLzntR/pDNPzntAlux), *E. coli* MC1061 (pSLcueR/pDNPcopAlux)	0.9% NaCl	< 100[c]	sphere	--	--	purchased from Sigma Aldrich	--	--	SEM	SEM, TEM	growth assay, luminescent bacterial test, luciferin-luciferase method	maximal growth rate, EC_{50}, EC_{99}, induction of bioluminescence, ATP content	combined ion-particle
15	*E. coli* K12 BW30270, *P. fluorescens* OS8	ultrapure water	11.6 ± 5.2	sphere	citrate (-25)	--	purchased from MK Nano (Missisauga, Canada)	--	--	DLS	single particle (SP)-ICP-MS for size, SEM-EDS for size and morphology, UV-vis	plating, ROS, and bioluminescence assays	CFU/mL, EC_{50}, ROS production, fold induction bioluminescence	combined ion-particle
			17.8 ± 8		citrate (-25)									
			47.7 ± 8		citrate (-24)									
			56.5 ± 9.6		citrate (-15)									
			94.8 ± 54		citrate (-16)									
16	*E. coli* AB1157, *E. coli* K12 MC 4100, *E. coli* K12 TK 821, *E. coli* K12 MH 1471, *E. coli* K12 MH 225, E. *coli* K12 MG1655 (pKatG::*lux*), *P. aeruginosa* PAO1, *P. chlororaphis 449, S. proteamaculans 94, S. liquefaciens MG1 and MG44*	Difco nutrient broth	8.3 ± 1.9	sphere	hydrolyzed casein peptides	--	prepared Ag oxide by adding $AgNO_3$ to 20% NaOH. Reduced Ag oxide with hydrolyzed casein peptides	--	--	SEM (one time point)	--	MIC assay, MTT assay, live/dead Baclight assay, biosensor assay, mutant growth assay	MIC, biofilm formation, bioluminescence, H_2O_2 concentration, gene regulation	combined ion-particle
17	*E. coli, S. aureus*	LB	6	sphere	--	--	prepared by the solvated metal atom dispersion method, which involves covaporization of Ag and solvent (acetone) onto a liquid N_2 cooled surface that warms.	--	--	TEM (one time point)	SEM/XRD/EDX for morphology, BET surface area	plate counting	number of surviving bacteria colonies	ion-only
					3-mercapto-1,2-propane-diol	yes – not toxic								
					sodium 3-mercapto-1-propane-	yes – not toxic								

					sulfonate, sodium 5-mercapto-1-tetra-zoleacetate	yes – not toxic	Ligands added and then heat treated, air exposed, and KOH exposed							
			1000 – 5000		Ag_2O	yes – not toxic	purchased from Aldrich							
					AgO	yes – not toxic								
18	*B. subtilis* UD1022, *E. coli* OP50	LB	44.9 ± 7.2	sphere [d]	citrate (-40.7)	yes – not toxic	purchased from Ted Pella Inc.	--	ICP-MS	DLS (duration)	--	growth assay, SEM	MIC/EC_{50}, growth kinetics, AgNP-cell interaction	combined ion-particle
19	nitrifying sludge	SWW	2 – 12	sphere	starch	--	purchased from Prime Nanotechnology Co, Ltd. (chemically synthesized from $AgNO_3$ using $NaBH_4$ and starch as reducing and capping agent)	--	--	EDX	UV-vis	SEM/TEM, ammonia oxidation, FISH, CLSM, live-dead Baclight assay,	AgNP-cell interaction, physiology and morphology change, maximum ammonia oxidation rate, % inhibition ammonia oxidation, % abundance, % membrane compromised	ion-only
20	*E. coli* DH5alpha DSMZ 6897, *S. aureus* DSMZ 1104	Lysogeny broth medium and Roswell Park Memorial Institute medium/10 % FCS medium	70 ± 20	sphere	PVP (-25)	--	reduced from $AgNO_3$ with glucose in the presence of PVP	ultracentrifugation	AAS	DLS	--	MIC/MBC assay	MIC/MBC	ion-only
21	*P. putida* mt-2 DSM 6125	mineral salt media with Na_2-succinate	< 10 [c]	sphere [e]	Tetra-decane	yes – not toxic	commercial AgNP dispersion (736503, Silverjet DGH-55LT-25C; Sigma-Aldrich)	--	--	--	--	growth assay, surface hydro-phobicity, analysis, fatty acid composition	growth inhibition, EC_{50}, water contact angle, cis/trans ratio/ membrane adaptive response	ion-only

22	PI-*Lac::GFP* (propidium iodide stained *E. coli* (*lac::GFP*))	modified LB without NaCl supplemented with ampicillin	10 40 100	sphere	--	--	manufactured by Sigma Aldrich Co. LLC	--	--	UV-vis, TEM (duration)	--	dual fluorescence flow cytometric analysis, effects of divalent metal ions, plate counting	ratio of damaged to living cells, response ratio of metal ions	combined ion-particle
23	*E. coli* MG1655	$NaHCO_3$	10.2 ± 2.3	sphere	citrate (-47.4)	--	synthesized using sodium citrate and tannic acid	ultrafiltration (10 kDa MWCO membrane; Millipore) and washed twice with deionized water	--	DLS (one time point)	X-ray binding energy analysis for surface characterization	SEM, NAD^+/NADH ratio test, intracellular ROS, malonyl-dialdehyde and 8-oxoguanine assay, Ag uptake and distribution evaluation	morphology, respiration chain disturbance, intracellular ROS production, lipid peroxidation, DNA damage, Ag uptake	combined ion-particle
			10.2 ± 2.5	sphere	MPA (-34.5)	--	ligand exchange with citrate AgNPs							
			10.2 ± 2.2	sphere	MHA (-29.1)	--								
			9.9 ± 2	sphere	MPS (-32.6)	--								
24	nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*)	agricultural soil of Toccoa sandy loam amended with a buffered nutrient solution ($(NH_4)_3PO_4$ and $(NH_4)_2SO_4$)	50	sphere[e]	--	--	purchased from Inframat Advanced Materials and Ultrasound Research Nanomaterials	--	--	--	--	batch soil-slurry nitrification method, sorption isotherm	nitrification kinetics (V_{max} nitrification potential), sorption	combined ion-particle
			15		PVP	--	purchased from Ultrasound Research Nanomaterials and Nanostructured and Amorphous Materials Inc.							
25	*S. oneidensis* MR-1	ferric citrate bacterial growth medium (minimized chloride content)	11 ± 3	sphere	citrate (-29 ± 3)	--	synthesized with trisodium citrate dihydrate and $NaBH_4$	centrifuged at 1500 g for 4 min using regenerated cellulose (MWCO 50 kDa) centrifugal filter units (EMD Millipore), resuspended in DI water, centrifuge/resuspension steps repeated 3X	UV-vis, ICP-MS	DLS (duration)	--	live/dead BacLight assay, TEM	live/dead ratio, morphology/ AgNP-cell interaction	ion-only
26	*P. putida* G7	phosphate buffer	50	sphere	-49.2 ± 1.32	--	purchased from Sigma-Aldrich	--	--	DLS (one time point)	BET surface area, TEM for surface characterization	ATP production (BacTiter-Glo) assay, computer-assisted motion analysis (phase	% viability, motility behavior, bacterial	particle-only

												contrast microscopy), chemical-in-pond method and inverted capillary assay		
27	*N. europaea* ATCC 19718	HEPES buffer and $(NH_4)_2SO_4$ minimal growth media	20 80	sphere	phosphate monolayer	--	synthesized through aqueous reduction by NanoComposix, Inc	--	ICP-ES	time-resolved (TR)-DLS (duration)	--	nitrite production and inhibition reversal colorimetric assay, membrane stability, AMO- and HAO-SOUR assay	% nitrification activity, % intracellular K^+, % SOUR activity	ion-only
28	*E. coli*	moderately hard water	10 ± 4.6	sphere	BPEI (+28.8)	yes – not toxic	synthesized with $AgNO_3$ and HEPES and UV radiation	tangential flow filtration system equipped with 10 kD hollow fiber polysulfone membranes; buffer exchange of impurities and ions in NP suspension with nanopure water	GF-AAS	DLS (before and after experiment)	UV-vis, PALS for zeta potential	*E. coli* bioassay	% inhibition of β-galactosidase enzyme, EC_{50}	particle-only
			56 ± 14		citrate (-20.08)	yes – not toxic	synthesized with $AgNO_3$ and sodium citrate dihydrate							
			72 ± 24		PVP (-7.49)	yes – not toxic	bought from Nanostructured and Amorphous Materials Inc. and added PVP							
29	*E. coli* MC1061 (pSLlux), *E. coli* MC1061 (pSLcueR/pDNcopAlux)	water purified with MilliQ equipment	83 ± 37	sphere	citrate (-36)	--	purchased from MKNano as aqueous suspension	--	GF-AAS	--	SEM-FIB-EDX for size and composition, DLS for HDD spheres, ELS for HDD for both shapes, UV-vis	bacterial biolumin-escence assay, bacterial viability plate assay	% inhibition of biolumin-escence, fold induction of biolumin-escence, EC_{50}	ion-only
			100 ± 40 (diameter) x 6100 ± 2700 (length)	nano-wire	-46	--	purchased from Seashell Technology as suspension in isopropanol							
30	Activated sludge	MLSS	5	sphere[e]	PEG	--	synthesized in another lab	--	--	--	--	SEM, community population shift, qPCR/pyrosequencing	activated sludge microstructure, % relative abundance, copy number of AMO	combined ion-particle
			35		carbon	--	purchased from Novaentrix Co.							
31	*E. coli* K12 TG1	distilled water, LB agar with ampicillin	52[b]	sphere	--	--	fabricated via reduction of $AgNO_3$ and hydroxylamine hydrochloride in NaOH solution	--	--	--	--	lumine-scence assay, polargraphic oxygen consumption method, AFM, cell	Biolumin-escence intensity (I), oxygen consumption rate, morphologic	ion-only

												viability serial dilution and plating assay	al changes, number of CFU, toxicity index (T)	
32	*E. coli* K12 MG 1655	LB, MH	24 ± 3.2	sphere	PEGSH (-11.2 +/- 1.5), ligand density: 1.46 ± 0.17 nm^{-2})	--	synthesized by aqueous reduction with $AgNO_3$, tannic acid, and citric acid. Ligand exchange to PEGSH	centrifuging and washing with DI water multiple times before and after ligand exchange with PEGSH	UV-vis, ICP-MS	TEM, DLS, UV-vis (duration)	NMR-TEM-ICP-MS for ligand density, XPS for AgCl formation	bacterial growth assay	OD_{600}	combined ion-particle

[a] Particle size determined by TEM/SEM
[b] DLS was used in place of TEM to determine particle size
[c] Particle size reported by manufacturer
[d] Included polygonal features (e.g., sharp corners)
[e] Assumed spherical shape

Abbreviations: AFM – atomic force microscopy; AMO – ammonia monooxygenase enzyme; AOB – ammonia oxidizing bacteria; ATP – adenosine triphosphate; BET – Brunauer-Emmett-Teller; BOD – biological oxygen demand; BPEI - branched poly(ethylene imine); CAPS – N-cyclohexyl-3-aminopropanesulfonic acid; CFU – colony forming unit; CLSM – confocal laser scanning microscopy; CPS – centrifugal particle sedimentation; DI – deionized water; DLS – dynamic light scattering; DNA – deoxyribonucleic acid; EC_{50} – effective concentration that affects 50% of the test population; ELS – electrophoretic light scattering; EDS/EDX – energy-dispersive x-ray spectroscopy; FBS – fetal bovine serum; FCS – fetal calf serum; FIB – focused ion beam; FISH – fluorescence in situ hybridization; FTIR – Fourier-transform infrared spectroscopy; GA – gum arabic; GF-AAS – graphite furnace atomic absorption spectroscopy; HAO – hydroxylamine oxidoreductase enzyme; HDD – hydrodynamic diameter; HEPES – (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); ICP-MS or OES – inductively coupled plasma mass spectrometry or optical emission spectroscopy; IC_{50}; inhibitory concentration that inhibits 50% of the test population; ISB – Iso-Sensitest broth; LB – Luria-Bertani; MBC – minimum bactericidal concentration; ME – microbiocidal effect; MH – Mueller-Hinton; MHA - mercaptohexanoic acid; MIC – minimum inhibitory concentration; MLC – minimum lethal concentration; MLSS – mixed liquor suspended solids; MPA - mercaptopropionic acid; MPS – mercaptopropionic sulfonic acid; MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromidefor; MWCO – molecular weight cut-off; NMR – nuclear magnetic resonance; OD – optical density; PALS – phase analysis light scattering; PBS – phosphate buffered saline; PEG - poly(ethylene glycol); PEGSH - poly(ethylene glycol) methyl ether thiol; PVP – poly(vinylpyrrolidone); ROS – reactive oxygen species; (RT) or (q)PCR – real-time or quantitative polymerase chain reaction; SEM – scanning electron microscopy; SOUR – specific oxygen uptake rate; SWW – synthetic wastewater; TEM – transmission electron microscopy; TGA – thermagravimetric analysis; TSB – Tryptone Soya broth; UV-vis – ultraviolet-visible spectroscopy; XPS – x-ray photoelectron spectroscopy; XRD – x-ray diffraction

Table S2. Analysis of bulk ion release reported and theoretically calculated, determination of a scaled ion control, and the resulting conclusion from each study in the final subset.

Refs	Method used to characterize dissolved Ag (filter and centrifugation settings)	Time point(s) measured (hr)	Solvent	Theoretical calculated percent Ag(I) release (%)	Reported = theoretically calculated dissolution? [a]	AgNP conc (mg/L)	Ag(I) ion conc (mg/L)	Form of Ag(I) ion	Assumed Ag(I) ion	mol/mL Ag as salt	mol/mL Ag as NP	Ratio of mol/mL Ag as salt:NP	Percent needing released to match ion	Scaled Ag(I) ion control present	Conclusion drawn

				(size (nm))								**control (%)**		
[3]	UV-vis	0, 24	DI water, LB	14.14 (9.1)	yes, full	1	1	$AgNO_3$	total Ag salt	5.89E-09	9.27E-09	16:25	64	no	particle-only, combined ion-particle
						100	10			5.89E-08	9.27E-07	8:125	6	yes	
				6.74 (19.1)		1	1			5.89E-09	9.27E-09	16:25	64	no	
						100	10			5.89E-08	9.27E-07	8:125	6	yes	
				2.96 (43.5)		1	1			5.89E-09	9.27E-09	16:25	63.50	no	
						100	10			5.89E-08	9.27E-07	8:125	6.35	yes	
				7.19 (17.9)		1	1			5.89E-09	9.27E-09	16:25	63.50	no	
						10	10			5.89E-08	9.27E-08	16:25	63.50	no	
				5.52 (23.3)		0.1	1			5.89E-09	9.27E-10	63:10	635.02	no	
						3	10			5.89E-08	2.78E-08	21:10	211.67	no	
				14.14 (9.1)		1.8	0.7			4.12E-09	1.67E-08	1:4	24.70	yes	
				6.74 (19.1)		8					7.42E-08	55:1000	5.56	yes	
				2.96 (43.5)		18.5					1.72E-07	3:125	2.40	yes	
				7.19 (17.9)		3.8					3.52E-08	3:25	11.70	yes	
				5.52 (23.3)		1.4					1.30E-08	8:25	31.75	yes	
[4]	ICP-OES/MS (cellulose membrane MWCO 10 kDa using a Amicon stir cell (Millipore) pressurized with	0.5, 240	AgNP stock solution	3.64 (35.4)	yes, partial	0.75	0.05	$AgNO_3$	total Ag atom of the Ag salt	4.64E-10	6.95E-9	7:100	6.67	yes	ion-only, combined ion-particle

	nitrogen)					6.2	0.3						4.84	yes	
5	ICP-MS	24, 48, 72, 96, 120	--, pH = 4	33.65 (3)	yes, partial	9	0.005	$AgNO_3$	total Ag atom of the Ag salt	4.64E-11	8.34E-08	3:5,000	0.06	yes	ion-only
						210	0.3			2.78E-09	1.95E-06	1:500	0.14	yes	
				25.74 (5)		9	0.005			4.64E-11	8.34E-08	3:5,000	0.06	yes	
						210	0.3			2.78E-09	1.95E-06	1:500	0.14	yes	
				11.70 (11)		9	0.005			4.64E-11	8.34E-08	3:5,000	0.06	yes	
						210	0.3			2.78E-09	1.95E-06	1:500	0.14	yes	
				7.15 (18)		10	0.005			4.64E-11	9.27E-08	5:10,000	0.05	yes	
						70	0.3			2.78E-09	6.49E-07	3:500	0.43	yes	
				2.52 (51)		10	0.005			4.64E-11	9.27E-08	5:10,000	0.05	yes	
						70	0.3			2.78E-09	6.49E-07	3:500	0.43	yes	
				1.79 (72)		10	0.005			4.64E-11	9.27E-08	5:10,000	0.05	yes	
						70	0.3			2.78E-09	6.49E-07	3:500	0.43	yes	
6	ICP-MS (centrifuged at 50,000 g for 10 min at 4C (Beckman-Coulter))	0.5, 1, 2, 4, 6, 8, 12, 24	LB lacking NaCl	0.91 (142)	yes, partial	200	0.4	$AgNO_3$	total Ag atom of the Ag salt	3.71E-09	1.85E-06	1:500	0.2	yes	combined ion-particle
						100	rate of AgNP ion release			--	9.27E-07	--	--	yes	
						500	1			9.27E-09	4.64E-06	1:500	0.2	yes	
						500	6			5.56E-08	4.64E-06	3:250	1.2	yes	
						1000	16			1.48E-07	9.27E-06	2:125	1.6	yes	
7	--	--	--	0.91 (142)	--	40	0.4	$AgNO_3$	total Ag atom of the Ag salt	3.71E-9	3.71E-7	1:100	1	yes	combined ion-particle

8	ICP-OES (Dialysis Tubing Benzoylated of nominal MWCO 2 kDa)	24	ISB	18.39 (7)	no	180	15	$AgNO_3$	total Ag salt	8.83E-08	1.67E-06	5:100	5.29	yes	particle-only
						15	10			5.89E-08	1.39E-07	2:5	42.33	no	
9	ICP-MS (dialysis cartridge float-a-lyzer G2 (Spectra/Por, Spectrum Laboratories, USA))	24, 48, 72	artificial sea-water and fresh-water	5.96 (20)	yes, full	0.005	0.02	$AgNO_3$	total Ag atom of the Ag salt	2E-10	5E-11	4:1	400	no	combined ion-particle
						2	0.5			5E-9	2E-08	1:4	25	yes	
				3.3 (40)		0.005	0.02			2E-10	5E-11	4:1	400	no	
						2	0.5			5E-9	2E-08	1:4	25	yes	
				1.32 (100)		0.005	0.02			2E-10	5E-11	4:1	400	no	
						2	0.5			5E-9	2E-08	1:4	25	yes	
				5.96 (20)		0.36	0.01			1E-10	3.30E-09	1:33	3.03	yes	
						1	0.2			2E-9	1E-08	1:5	20	yes	
				3.3 (40)		0.36	0.01			1E-10	3.30E-09	1:33	3.03	yes	
						1	0.2			2E-9	1E-08	1:5	20	yes	
				1.32 (100)		0.36	0.01			1E-10	3.30E-09	1:33	3.03	yes	
						1	0.2			2E-9	1E-08	1:5	20	no	
10	ICP-MS (Amicon Ultra-4 Centrifugal Filter Unit 7,000 rpm 35 min, <3 kDa pore size)	0, 24	AOB media	5.11 (25.2)	no	0.2	0.2	$AgNO_3$	total Ag atom of the Ag salt	1.85E-09	1.85E-09	1:1	100	no	ion-only
				4.77 (27)		2	2			1.85E-08	1.85E-08	1:1	100	no	
							0.2			1.85E-09	1.85E-08	1:10	10	yes	
				6.13 (21)		20	0.2			1.85E-09	1.85E-07	1:100	1	yes	
11	AAS, Orion 013010MD conductivity cell, ultrafiltration membrane 10 kDa polyethersulfone (MidiKros Hollow Fiber Module (P-X3-010E-300-02N))	over 3 weeks	purified AgNP stock solution, BOD5 test media	2.22 (58)	yes, partial	0.003	0.003	$AgNO_3$	total Ag atom of the Ag salt	2.78E-11	2.78E-11	1:1	100	no	particle-only
						0.3	0.070			6.49E-10	2.78E-09	23:100	23.33	yes	
				6.77 (19)		0.3	0.003			2.78E-11	2.78E-11	1:1	100	no	
						1	0.070			6.49E-10	9.27E-09	7:100	7	yes	
				10.73 (12)		0.003	0.003			2.78E-11	2.78E-11	1:1	100	no	

						0.3	0.070			6.49E-10	2.78E-09	23:100	23.33	yes	
				12.87 (10)		0.003	0.003			2.78E-11	2.78E-11	1:1	100	no	
						0.3	0.070			6.49E-10	2.78E-09	23:100	23.33	yes	
12	--	--	--	6.34 (20.3)	--	5	0.25	$AgNO_3$	total Ag salt	1.47E-09	4.64E-08	3:100	3.18	yes	ion-only
						50	2			1.18E-08	4.64E-07	1:40	2.54	yes	
				2.42 (53.1)		10	0.25			1.47E-09	9.27E-08	2:125	1.59	yes	
						100	2			1.18E-08	9.27E-07	3:250	1.27	yes	
				1.14 (112.6)		50	0.25			1.47E-09	4.64E-07	3:1000	0.32	yes	
						220	2			1.18E-08	2.04E-06	6:1000	0.58	yes	
13	ICP-MS, 40 min at 4000 g using centrifugal filter devices (Amicon ultra-4, Millipore, Ireland) with 3 kDa MWCO	0.5, 6, 24, 48, 72	2% NaCl, NBBM, Volvic water	6.44 (20)	yes, partial	0.1	0.05	$AgNO_3$	total Ag salt	2.94E-10	9.27E-10	8:25	31.75	yes	combined ion-particle
				0.64 (200)		300	7			4.12E-08	2.78E-06	15:1000	1.48	yes	
				5.60 (23)		0.05	0.05			2.94E-10	4.64E-10	63:100	63.50	no	
				4.77 (27)		100	7			4.12E-08	9.27E-07	2:45	4.45	yes	
14b	bioluminescence	0.5, 2	0.9% NaCl	1.29 (100)	no	0.01	0.01	$AgNO_3$	total Ag salt	5.89E-11	9.27E-11	63:100	63.50	no	combined ion-particle
						1000	10			5.89E-08	9.27E-06	63:10,000	0.64	yes	
						0.001	0.0002			1.18E-12	9.27E-12	13:100	12.70	yes	
						3	0.2			1.18E-09	2.78E-08	21:500	4.23	yes	
15	Single particle (SP)-ICP-MS and GF-AAS (ultracentrifugation at 390,000 g for 30 min (Beckman-Coulter	4, 24, 48, 72	Ultra-pure water, artificial fresh-water, algal	11.1 (11.6)	yes, partial	0.01	0.0001	$AgNO_3$	total Ag salt	5.89E-13	9.27E-11	4:625	0.64	yes	combined ion-particle
						10	0.04			2.35E-10	9.27E-08	25:10,000	0.25	yes	
				7.23 (17.8)		0.01	0.0001			5.89E-13	9.27E-11	4:625	0.64	yes	

	ultracentrifuge L8-55 M))		media, cell culture media			10	0.04			2.35E-10	9.27E-08	25:10,000	0.25	yes	
				2.7 (47.7)		0.01	0.0001			5.89E-13	9.27E-11	4:625	0.64	yes	
						10	0.04			2.35E-10	9.27E-08	25:10,000	0.25	yes	
				2.28 (56.5)		0.01	0.0001			5.89E-13	9.27E-11	4:625	0.64	yes	
						10	0.04			2.35E-10	9.27E-08	25:10,000	0.25	yes	
				1.36 (94.8)		0.01	0.0001			5.89E-13	9.27E-11	4:625	0.64	yes	
						10	0.04			2.35E-10	9.27E-08	25:10,000	0.25	yes	
16	--	--	--	15.51 (8.3)	--	0.125	0.0625	$AgNO_3$	total Ag atom of the Ag salt	5.79E-10	1.16E-09	1:2	50	no	combined ion-particle
						64	2			1.85E-08	5.93E-07	3:100	3.13	yes	
17	conductivity	--	--	21.45 (6)	--	37.5	3.75	$AgNO_3$	total Ag salt	2.21E-08	3.48E-07	3:50	6.35	yes	ion-only
						375	37.5			2.21E-07	3.48E-06	3:50	6.35	yes	
						3750	375			2.21E-06	3.48E-05	3:50	6.35	Yes	
18	ICP-MS, filtered through a 0.025-µm polyethersulfone membrane (Millipore, Billerica, MA, USA) and digested for 24 hrs	48-168	DI water, LB, PNS+RE	2.87 (44.9)	yes, full	0.1	0.1	$AgNO_3$	total Ag salt	5.89E-10	9.27E-10	3:5	63.50	no	combined ion-particle
						1					9.27-09	3:50	6.35	yes	
						5					4.64E-08	1:80	1.27	yes	
						0.1	1			5.89E-09	9.27E-10	635:100	635.02	no	
						1					9.27E-09	635:1000	63.50	no	
						5					4.64E-08	127:1000	12.70	yes	
19	AAS, ultracentrifuge machine (Optima™ MAX-XP Ultracentrifuge, Beckman Coulter, Inc., USA) and	0, 30, 60	inorganic SWW, SWW with nitrifying sludge	18.39 (7)	no	1	0.05	$AgNO_3$	total Ag atom of the Ag salt	4.64E-10	9.27E-09	1:20	5	yes	ion-only

	centrifuged at 165000×g, 4 °C, for 2 h using MLN-80 rotor (16 × 58 mm, Beckman Coulter, Inc., USA)					10	0.10			9.27E-10	9.27E-08	1:100	1	yes	
						100	0.50			4.64E-09	9.27E-07	1:200	0.5	yes	
						100	5			4.64E-08	9.27E-07	1:20	5	yes	
20	--	--	--	1.84 (70)	--	12.5	1	Ag-$C_2H_3O_2$	total Ag atom of the Ag salt	9.27E-09	1.16E-07	1:11	8	yes	ion-only
						50	10			9.27E-08	4.64E-07	1:5	20	yes	
21	--	--	--	12.87 (10)	no	200	0.05	$AgNO_3$	total Ag salt	2.94E-10	1.85E-06	1:6300	0.02	Yes	ion-only
						900	0.3			1.77E-09	8.34E-06	1:4700	0.02	Yes	
22	UV-vis/ICP-MS	--	LB lacking NaCl with ampicillin	12.87 (10)	yes, full	0.2	--	--	total Ag atom of the Ag salt	--	--	--	--	--	combined ion-particle
				3.22 (40)		0.4	0.04			3.71E-10	3.71E-09	1:10	10	yes	
				1.29 (100)		0.6	--			--	--	--	--	--	
23	ICP-MS, ultrafiltration with 3kDa MWCO membrane Millipore	6, 24	$NaHCO_3$	12.87 (10)	no	1	0.005	$AgNO_3$	total Ag atom of the Ag salt	4.64E-11	9.27E-09	1:200	0.5	yes	combined ion-particle
						5	0.02			1.85E-10	4.64E-08	1:250	0.4	yes	
						5	0.05			4.64E-10	4.64E-08	1:100	1	yes	
						15	0.2			1.85E-09	1.39E-07	2:150	1.33	yes	
						15	0.5			4.64E-09	1.39E-07	1:30	3.33	yes	
						15	1			9.27E-09	1.39E-07	1:15	6.67	yes	
24	ICP-AES; filtered by centrifugation using microfilter	24, 48, 72, 96, 120	buffered nutrient solution	2.57 (50)	yes, partial	1	1	Ag_2SO_4	total Ag atom of the Ag salt	9.27E-09	9.27E-09	1:1	100	no	combined ion-particle
						300	10			9.27E-08	2.78E-06	1:30	3.33	yes	

	centrifuge tubes (washed with copper nitrate) at 3,750 g for 20 minutes					50	5			4.64E-08	4.64E-07	1:10	10	yes	
						100	10			9.27E-08	9.27E-07	1:10	10	yes	
				8.58 (15)		1	1			9.27E-09	9.27E-09	1:1	100	no	
						10	10			9.27E-08	9.27E-08	1:1	100	no	
						50	5			4.64E-08	4.64E-07	1:10	10	yes	
						100	10			9.27E-08	9.27E-07	1:10	10	yes	
25	Ag+ selective electrodes with ionophore-doped fluorous sensing membrane, ICP-MS, in presence and absence of bacteria	over 24	DI water	11.70 (11)	yes, full	3	0.3	--	total Ag atom of the Ag salt	3.00E-09	2.78E-08	1:9	10.79	yes	ion-only
						0.3	0.1			1.00E-09	2.78E-09	4:11	35.96	yes	
						15	1			1.00E-08	1.39E-07	7:100	7.19	yes	
26	--	--	--	2.57 (50)	--	0.2	0.001	$AgNO_3$	total Ag salt	5.89E-12	1.85E-09	1:300	0.32	yes	particle-only
						0.2	0.01			5.89E-11	1.85E-09	1:30	3.18	yes	
						0.2	0.1			5.89E-10	1.85E-09	1:3	31.75	yes	
						0.1	0.1			5.89E-10	9.27E-10	5:8	63.50	no	
						1	1			5.89E-9	9.27E-9	5:8	63.50	no	
						100	100			5.89E-07	9.27E-07	5:8	63.50	no	
				1.29 (100)		0.2	0.001			5.89E-12	1.85E-09	1:300	0.32	yes	
						0.2	0.01			5.89E-11	1.85E-09	1:30	3.18	yes	
						0.2	0.1			5.89E-10	1.85E-09	1:3	31.75	yes	
						0.1	0.1			5.89E-	9.27E-	5:8	63.50	no	

										10	10				
						1	1			5.89E-9	9.27E-9	5:8	63.50	no	
						100	100			5.89E-07	9.27E-07	5:8	63.50	no	
27	ICP-ES, centrifugal ultra-filtration device with 5 kDa MWCO (Amicon Ultra-15, Millipore Co., Billericka, MA), 30 min at 5000g	3	HEPES buffer, minimal media	6.44 (20)	yes, partial	0.30	0.05	$AgNO_3$	total Ag atom of the Ag salt	4.64E-10	2.78E-09	1:6	16.67	yes	ion-only
						0.75	0.05			4.64E-10	6.95E-09	2:30	6.67	yes	
						0.75	1.25			1.16E-08	6.95E-09	83:50	166.67	no	
						10	1			9.27E-09	9.27E-08	1:10	10	yes	
				1.61 (80)		2.75	0.2			1.85E-09	2.55E-08	1:14	7.27	yes	
28	GF-AAS, centrifuged for 30 min at ~3150 g (4000 rpm, Thermo Electron, IEC Centra CL3 Series Centrifuge)	48	--	12.87 (10)	yes, full	0.01	0.04	$AgNO_3$	total Ag atom of the Ag salt	3.71E-10	9.27E-11	4:1	400	no	particle-only
						8.7	3.62			3.36E-08	8.07E-08	2:5	41.61	no	
				2.3 (56)		0.02	0.04			3.71E-10	1.85E-10	2:1	200	no	
						13.2	3.62			3.36E-08	1.22E-07	7:27	27.42	yes	
				1.79 (72)		0.05	0.04			3.71E-10	4.64E-10	4:5	80	no	
						41.2	3.62			3.36E-08	3.82E-07	9:100	8.79	yes	
29	AAS or Ag-sensor bacteria, ultracentrifuged at 390 000 g for 45 minutes	4	--	1.55 (83, sphere)	yes, partial	0.01	0.0003	$AgNO_3$	total Ag atom of the Ag salt	2.78E-12	9.27E-11	3:100	3	yes	ion-only
						30	0.5			4.64E-09	2.78E-07	2:125	1.67	yes	
				0.82 (100 X 6100, wire)		0.01	0.0003			2.78E-12	9.27E-11	3:100	3	yes	
						30	0.5			4.64E-09	2.78E-07	2:125	1.67	yes	
30	ICP-MS, ultracentrifugation at 35,000 rpm for 4 hr	--	--	25.74 (5)	--	0.05	1	$AgNO_3$	total Ag atom of the Ag salt	9.27E-09	4.64E-10	20:1	2000	no	combined ion-particle

				3.68 (35)		40	1			9.27E-09	3.71E-07	1:40	2.5	yes	
31	--	--	--	2.48 (52)	--	3.4	0.005	$AgNO_3$	total Ag salt	2.94E-11	3.15E-08	9:10,000	0.09	Yes	ion-only
						34	0.1			5.89E-10	3.15E-07	19:10,000	0.19	Yes	
						8.5	0.02			1.18E-10	7.88E-08	15:10,000	0.15	Yes	
						44	11			6.48E-08	4.08E-07	8:50	15.88	Yes	
						22	0.22			1.30E-09	2.04E-07	63:10,000	0.64	Yes	
32	ICP-MS, centrifuged at 20000 rcf for 20 min	0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24	LB, MH	5.36 (24)	yes, full	6.7	rate of AgNP ion release	$AgNO_3$	total Ag atom of the Ag salt	--	6.21E-08	--	--	yes	combined ion-particle

[a] Full = entire range of theoretical values is within the reported measured concentration range of Ag(I) ions, partial = only a portion of the range of theoretical values is within the reported measured concentration range of Ag(I) ions, no = there is no overlap in the range of theoretical values and the reported measured concentration range of Ag(I) ions (see Figure S2).

[b] Intracellular dissolution was used in place of bulk dissolution since it was the only value reported.

Abbreviations: AOB – ammonia oxidizing bacteria; BOD – biological oxygen demand; DI – deionized water; GF-AAS – graphite furnace atomic absorption spectroscopy; HEPES – (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); ICP-MS or OES or AES – inductively coupled plasma mass spectrometry or optical or atomic emission spectroscopy; LB – Luria-Bertani; MH – Mueller-Hinton; MWCO – molecular weight cut-off; NBBM – Bold's Basal Medium enriched with nitrate; PEGSH - poly(ethylene glycol) methyl ether thiol; PNE+RE – Plant nutrient solution containing *Z. mays* root excretion; SWW – synthetic wastewater; UV-vis – ultraviolet-visible spectroscopy

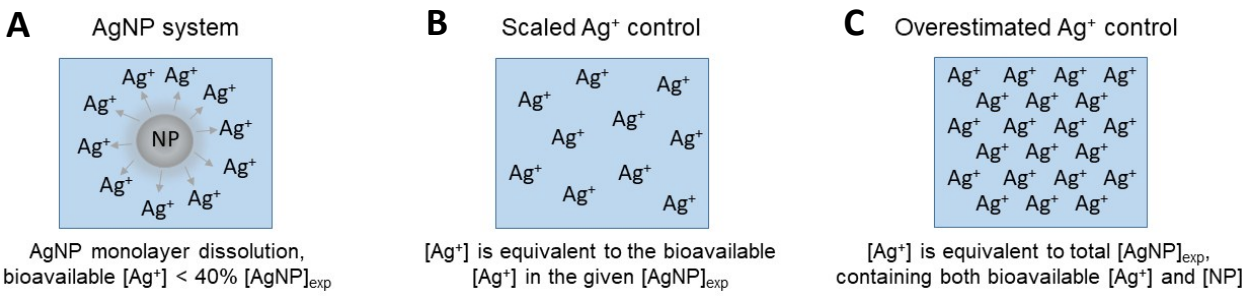

Figure S1. Schematic demonstrating the difference between an appropriate and inappropriate ion control. (A) The AgNP system contains the zero-valent NP and dissolved Ag(I) ions. (B) An appropriate ion control contains dissolved silver at an equivalent concentration to the Ag(I) ions released from the AgNP. (C) A concentration of dissolved silver equivalent to the total silver present in the AgNP would not serve as an appropriate ion control.

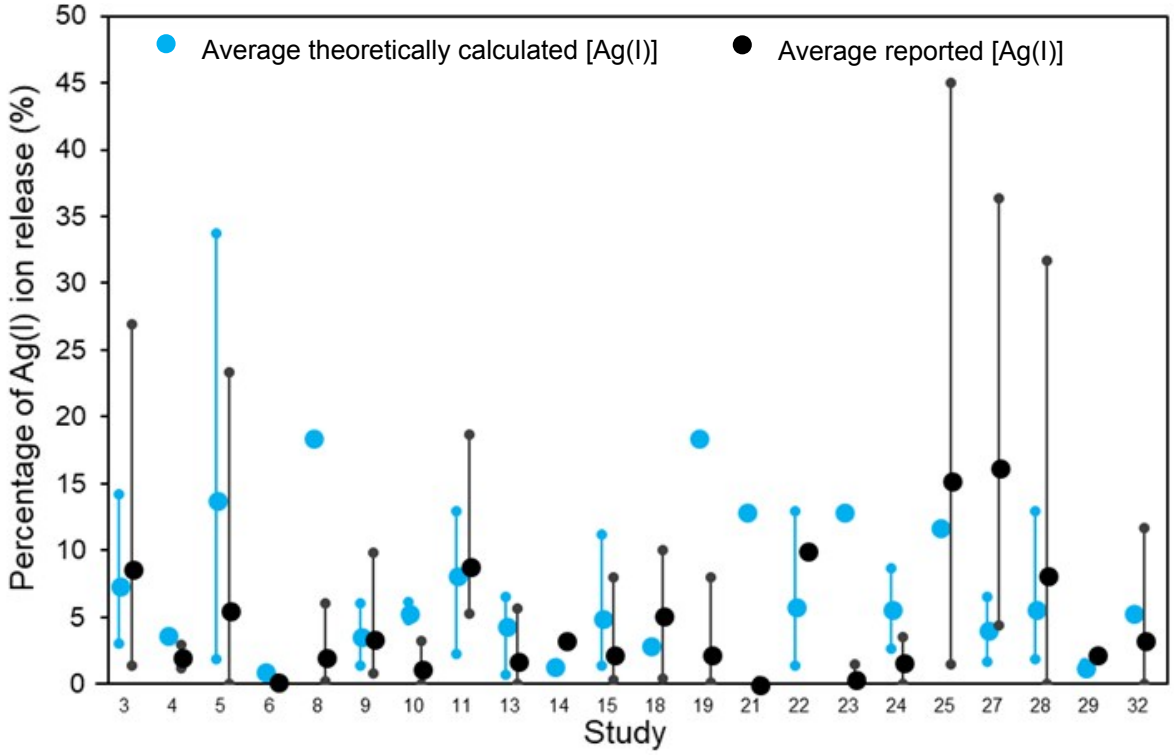

Figure S2. Comparison of the reported dissolution values in studies that measured dissolution to our theoretical calculations of dissolution. The middle data points represent the average dissolution value with the upper and lower data points representing the maximum and minimum dissolution values, respectively. Dissolution reported by the majority of studies (73%) align fully or partially with our theoretically calculated dissolution. Thus, the agreement between theoretical dissolution values and average reported dissolution values for the majority of studies further validates our calculation approach to determining an appropriate ion control. Note: The range for the reported dissolution includes different particle sizes, concentrations of AgNPs, different techniques to measure dissolution, and different media. The range for the theoretical calculations include different particle sizes and shapes.

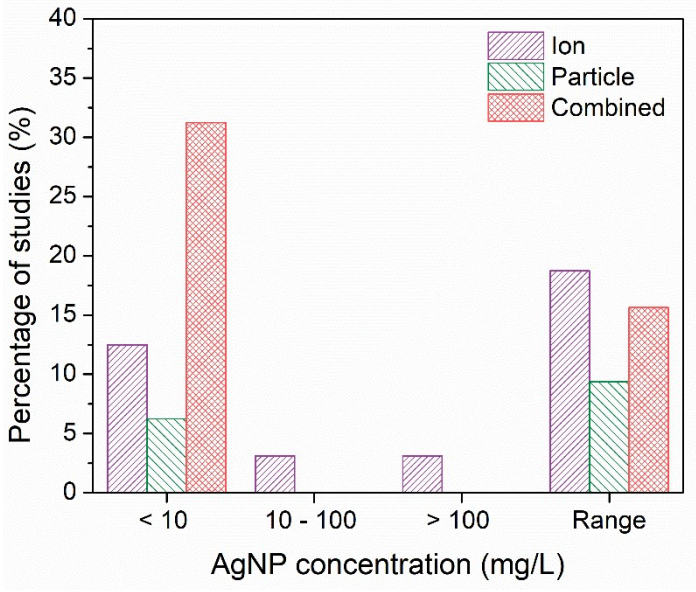

Figure S3. Ion-only, particle-only, and combined ion-particle conclusions drawn from studies that expose bacteria to <10 mg/L,[4, 6-9, 11, 15, 16, 18, 22, 23, 25, 27, 31, 32] 10-100 mg/L,[20] >100 mg/L,[21] and a range[3, 5, 10, 12-14, 17, 19, 24, 26, 28-30] of AgNP concentrations. No meaningful conclusion can be discerned.

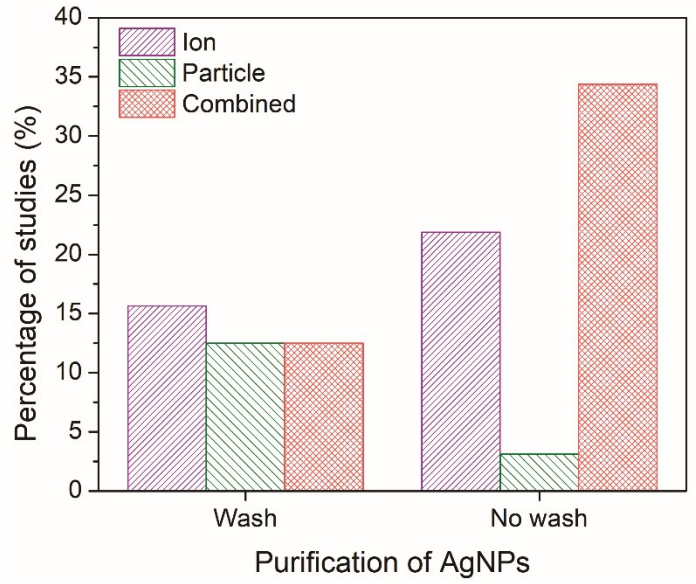

Figure S4. Ion-only, particle-only, and combined ion-particle conclusions drawn from studies that did[3, 4, 8, 10-12, 20, 23, 25, 28, 32] or did not[5-7, 9, 13-19, 21, 22, 24, 26, 27, 29-31] purify AgNPs post-synthesis. No meaningful conclusion can be discerned.

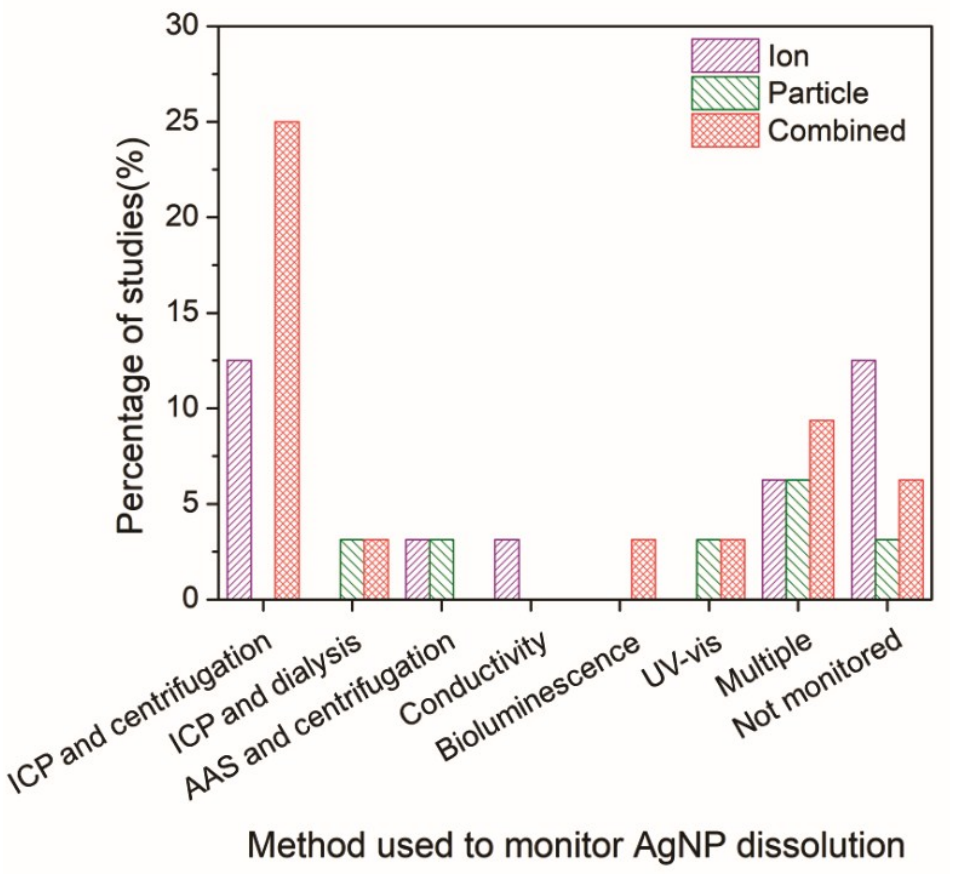

Figure S5. Ion-only, particle-only, and combined ion-particle conclusions drawn from studies that used ICP and centrifugation,[4-6, 10, 13, 18, 23, 24, 27, 30, 32] ICP and dialysis[8, 9] AAS and centrifugation,[19, 28] conductivity,[17] bioluminescence,[14] multiple methods,[3, 11, 15, 22, 25, 29] and no methods[7, 12, 16, 20, 21, 26, 31] to monitor dissolution. No meaningful conclusion can be discerned.

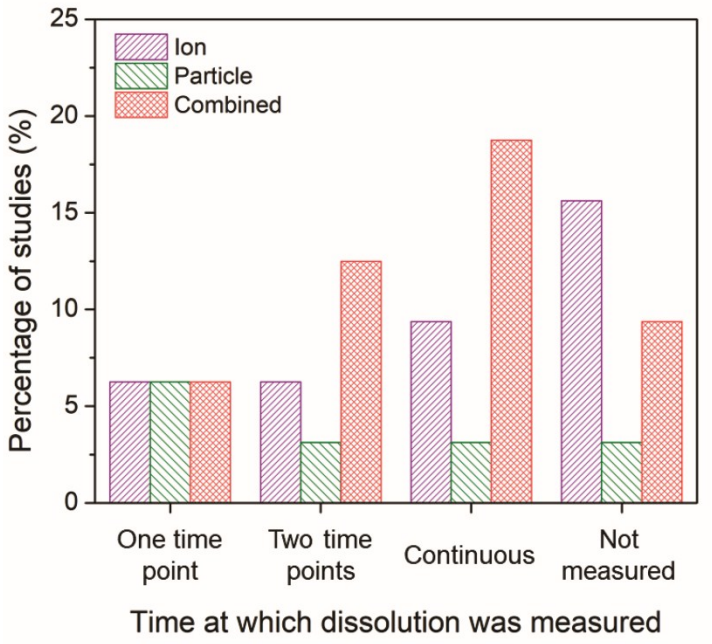

Figure S6. Ion versus particle conclusions drawn from studies that measure ion release at one time point,[8, 13, 22, 27-29] two time points,[3, 4, 10, 14, 23] continuously,[5, 6, 9, 11, 15, 18, 19, 24, 25, 32] or not at all[7, 12, 16, 17, 20, 21, 26, 30, 31]. Measuring dissolution is important for observing particle-only and combined ion-particle effects, especially at multiple time points.

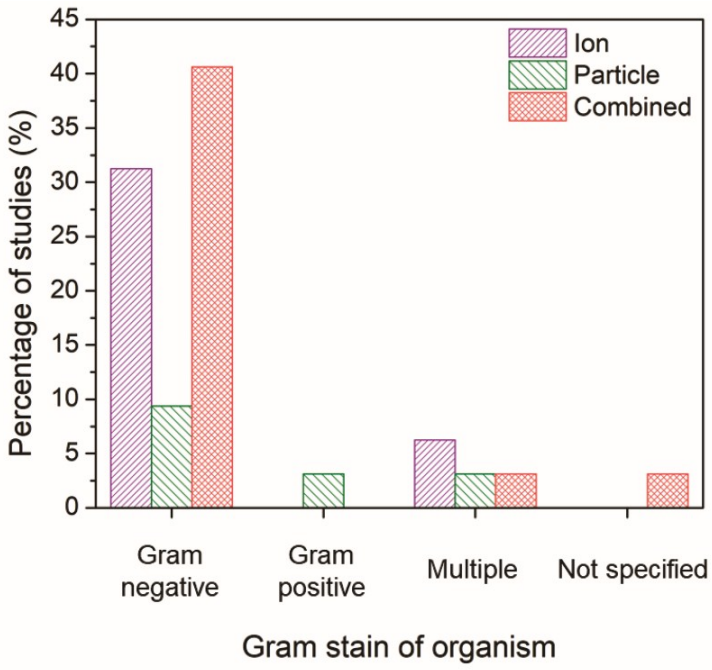

Figure S7. Ion-only, particle-only, and combined ion-particle conclusions drawn from studies that expose AgNPs and Ag(I) ions to only gram negative,[3-7, 9, 10, 12-16, 19, 21-29, 31, 32] gram positive,[11] both types,[8, 17, 18, 20] or unspecified[30] bacteria. No meaningful conclusion can be discerned.

References

1. H. Hofmeister and G. L. Tan, Shape and internal structure of silver nanoparticles embedded in glass, *Journal of Materials Research*, 2005, **20**, 1551-1562.
2. S. Pal, Y. K. Tak and J. M. Song, Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium Escherichia coli, *Appl Environ Microbiol*, 2007, **73**, 1712-1720.
3. A. Ivask, A. Elbadawy, C. Kaweeteerawat, D. Boren, H. Fischer, Z. Ji, C. H. Chang, R. Liu, T. Tolaymat, D. Telesca, J. I. Zink, Y. Cohen, P. A. Holden and H. A. Godwin, Toxicity mechanisms in Escherichia coli vary for silver nanoparticles and differ from ionic silver, *ACS Nano*, 2014, **8**, 374-386.
4. Z. M. Xiu, J. Ma and P. J. Alvarez, Differential effect of common ligands and molecular oxygen on antimicrobial activity of silver nanoparticles versus silver ions, *Environ Sci Technol*, 2011, **45**, 9003-9008.
5. Z. M. Xiu, Q. B. Zhang, H. L. Puppala, V. L. Colvin and P. J. Alvarez, Negligible particle-specific antibacterial activity of silver nanoparticles, *Nano Lett*, 2012, **12**, 4271-4275.
6. J. S. McQuillan, H. G. Infante, E. Stokes and A. M. Shaw, Silver nanoparticle enhanced silver ion stress response in Escherichia coli K12, *Nanotoxicology*, 2012, **6**, 857-866.
7. J. S. McQuillan and A. M. Shaw, Differential gene regulation in the Ag nanoparticle and Ag(+)-induced silver stress response in Escherichia coli: a full transcriptomic profile, *Nanotoxicology*, 2014, **8 Suppl 1**, 177-184.
8. E. Amato, Y. A. Diaz-Fernandez, A. Taglietti, P. Pallavicini, L. Pasotti, L. Cucca, C. Milanese, P. Grisoli, C. Dacarro, J. M. Fernandez-Hechavarria and V. Necchi, Synthesis, characterization and antibacterial activity against Gram positive and Gram negative bacteria of biomimetically coated silver nanoparticles, *Langmuir*, 2011, **27**, 9165-9173.
9. A. D. Burchardt, R. N. Carvalho, A. Valente, P. Nativo, D. Gilliland, C. P. Garcia, R. Passarella, V. Pedroni, F. Rossi and T. Lettieri, Effects of silver nanoparticles in diatom Thalassiosira pseudonana and cyanobacterium Synechococcus sp, *Environ Sci Technol*, 2012, **46**, 11336-11344.
10. C. L. Arnaout and C. K. Gunsch, Impacts of silver nanoparticle coating on the nitrification potential of Nitrosomonas europaea, *Environ Sci Technol*, 2012, **46**, 5387-5395.
11. A. M. El Badawy, R. G. Silva, B. Morris, K. G. Scheckel, M. T. Suidan and T. M. Tolaymat, Surface Charge-Dependent Toxicity of Silver Nanoparticles, *Environmental Science and Technology*, 2011, **45**, 283-287.
12. C. R. Bowman, F. C. Bailey, M. Elrod-Erickson, A. M. Neigh and R. R. Otter, Effects of silver nanoparticles on zebrafish (Danio rerio) and Escherichia coli (ATCC 25922): a comparison of toxicity based on total surface area versus mass concentration of particles in a model eukaryotic and prokaryotic system, *Environ Toxicol Chem*, 2012, **31**, 1793-1800.
13. A. Georgantzopoulou, Y. L. Balachandran, P. Rosenkranz, M. Dusinska, A. Lankoff, M. Wojewodzka, M. Kruszewski, C. Guignard, J. N. Audinot, S. Girija, L. Hoffmann and A. C. Gutleb, Ag nanoparticles: size- and surface-dependent effects on model aquatic organisms and uptake evaluation with NanoSIMS, *Nanotoxicology*, 2013, **7**, 1168-1178.
14. A. Ivask, O. Bondarenko, N. Jepihhina and A. Kahru, Profiling of the reactive oxygen species-related ecotoxicity of CuO, ZnO, TiO2, silver and fullerene nanoparticles using a set of recombinant luminescent Escherichia coli strains: differentiating the impact of particles and solubilised metals, *Anal Bioanal Chem*, 2010, **398**, 701-716.
15. A. Ivask, I. Kurvet, K. Kasemets, I. Blinova, V. Aruoja, S. Suppi, H. Vija, A. Kakinen, T. Titma, M. Heinlaan, M. Visnapuu, D. Koller, V. Kisand and A. Kahru, Size-dependent toxicity of silver

nanoparticles to bacteria, yeast, algae, crustaceans and mammalian cells in vitro, *PLoS One*, 2014, **9**, e102108.
16. M. A. Radzig, V. A. Nadtochenko, O. A. Koksharova, J. Kiwi, V. A. Lipasova and I. A. Khmel, Antibacterial effects of silver nanoparticles on gram-negative bacteria: influence on the growth and biofilms formation, mechanisms of action, *Colloids Surf B Biointerfaces*, 2013, **102**, 300-306.
17. A. B. Smetana, K. J. Klabunde, G. R. Marchin and C. M. Sorensen, Biocidal activity of nanocrystalline silver powders and particles, *Langmuir*, 2008, **24**, 7457-7464.
18. M. A. Doody, D. Wang, H. P. Bais and Y. Jin, Differential antimicrobial activity of silver nanoparticles to bacteria Bacillus subtilis and Escherichia coli, and toxicity to crop plant Zea mays and beneficial B. subtilis-inoculated Z. mays, *Journal of Nanoparticle Research*, 2016, **18**, 290.
19. N. T. Giao, T. Limpiyakorn, P. Kunapongkiti, P. Thuptimdang and S. Siripattanakul-Ratpukdi, Influence of silver nanoparticles and liberated silver ions on nitrifying sludge: ammonia oxidation inhibitory kinetics and mechanism, *Environ Sci Pollut Res Int*, 2017, **24**, 9229-9240.
20. C. Greulich, D. Braun, A. Peetsch, J. Diendorf, B. Siebers, M. Epple and M. Koller, The toxic effect of silver ions and silver nanoparticles towards bacteria and human cells occurs in the same concentration range, *RSC Advances*, 2012, **2**, 6981-6987.
21. N. Hachicho, P. Hoffmann, K. Ahlert and H. J. Heipieper, Effect of silver nanoparticles and silver ions on growth and adaptive response mechanisms of Pseudomonas putida mt-2, *FEMS Microbiol Lett*, 2014, **355**, 71-77.
22. W. Hong, L. Li, J. Liang, J. Wang, X. Wang, S. Xu, L. Wu, G. Zhao, A. Xu and S. Chen, Investigating the environmental factors affecting the toxicity of silver nanoparticles in Escherichia coli with dual fluorescence analysis, *Chemosphere*, 2016, **155**, 329-335.
23. Y. M. Long, L. G. Hu, X. T. Yan, X. C. Zhao, Q. F. Zhou, Y. Cai and G. B. Jiang, Surface ligand controls silver ion release of nanosilver and its antibacterial activity against Escherichia coli, *Int J Nanomedicine*, 2017, **12**, 3193-3206.
24. A. Masrahi, A. R. VandeVoort and Y. Arai, Effects of silver nanoparticle on soil-nitrification processes, *Arch Environ Contam Toxicol*, 2014, **66**, 504-513.
25. M. A. Maurer-Jones, M. P. S. Mousavi, L. D. Chen, P. Buhlmann and C. L. Haynes, Characterization of silver ion dissolution from silver nanoparticles using fluorous-phase ion-seelctive electrodes and assessment of resultant toxicity to Shewanella oneidensis, *Chemical Science*, 2013, **4**, 2564-2572.
26. J. J. Ortega-Calvo, R. Molina, C. Jimenez-Sanchez, P. J. Dobson and I. P. Thompson, Bacterial tactic response to silver nanoparticles, *Environ Microbiol Rep*, 2011, **3**, 526-534.
27. T. S. Radniecki, D. P. Stankus, A. Neigh, J. A. Nason and L. Semprini, Influence of liberated silver from silver nanoparticles on nitrification inhibition of Nitrosomonas europaea, *Chemosphere*, 2011, **85**, 43-49.
28. T. Silva, L. R. Pokhrel, B. Dubey, T. M. Tolaymat, K. J. Maier and X. Liu, Particle size, surface charge and concentration dependent ecotoxicity of three organo-coated silver nanoparticles: comparison between general linear model-predicted and observed toxicity, *Sci Total Environ*, 2014, **468-469**, 968-976.
29. M. Visnapuu, U. Joost, K. Juganson, K. Kunnis-Beres, A. Kahru, V. Kisand and A. Ivask, Dissolution of silver nanowires and nanospheres dictates their toxicity to Escherichia coli, *Biomed Res Int*, 2013, **2013**, 819252.
30. Y. Yang, J. Quensen, J. Mathieu, Q. Wang, J. Wang, M. Li, J. M. Tiedje and P. J. Alvarez, Pyrosequencing reveals higher impact of silver nanoparticles than Ag+ on the microbial community structure of activated sludge, *Water Res*, 2014, **48**, 317-325.

31. A. P. Zarubina, L. I. Deev, I. M. Parkhomenko, E. Y. Parshina, A. S. Sarycheva, L. A. Novoselova, E. P. Lukashev, A. I. Netrusov and A. B. Rubin, Evaluation of Toxicity of Silver Ions and Nanoparticles using Model Bacteria with Luminescent Phenotype, *Nanotechnologies in Russia*, 2015, **10**, 475-483.
32. K. A. Johnston, L. M. Stabryla, A. M. Smith, X. Y. Gan, L. M. Gilbertson and J. E. Millstone, Impacts of broth chemistry on silver ion release, surface chemistry composition, and bacterial cytotoxicity of silver nanoparticles, *Environmental Science: Nano*, 2018, **5**, 304-312.