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Supplementary Information

2 **Antibacterial Mechanism of Silver Nanoparticles in *Pseudomonas*** 3 ***aeruginosa*: Proteomics Approach**

4 Xueting Yan, †, ‡ Bin He, †, ‡, * Lihong Liu, †, ‡ Guangbo Qu, †, ‡ Jianbo Shi, †, ‡ Ligang Hu, †,
5 §, * Guibin Jiang†, ‡

6 †State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center
7 for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

8 ‡College of Resources and Environment, University of Chinese Academy of Sciences,
9 Beijing, 100049, China

10 §Institute of Environment and Health, Jiangnan University, Wuhan, Hubei 430056, China

11 **Corresponding author:** *Ligang Hu, *Bin He

12 **Email:** lghu@rcees.ac.cn; bhe@rcees.ac.cn

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14 The supplementary material contains twelve pages, with three experimental methods,
15 four tables and three figures.

16 The experimental methods contain chemicals, measurement of silver ion release, I-
17 markers synthesis, gel preparation and ICP-MS detection.

18 **Tables and Figures**

19 Table S1. Differentially expressed proteins identified in *P. aeruginosa* from AgNPs (1.2
20 $\mu\text{g mL}^{-1}$) exposure.

21

22 Table S2. Differentially expressed proteins identified in *P. aeruginosa* from AgNO₃ (0.6
23 $\mu\text{g mL}^{-1}$) exposure.

24

25 Table S3. Identified silver-binding proteins from *P. aeruginosa*.

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27 Table S4. ICP-MS operating conditions.

28

29 Fig. S1 TEM image of 5 nm AgNPs. An aliquot of sonicated AgNPs suspension was
30 dispersed on the carbon membrane coated copper net and dried at room temperature.

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32 Fig. S2 Release of silver ions from AgNPs after 12 h and 24 h. The initial concentrations
33 of total silver content were 0.1, 0.2, 0.4, and 0.6 $\mu\text{g mL}^{-1}$, respectively. Significantly
34 different from control: * $p < 0.05$.

35

36 Fig. S3 Major Gene ontology (GO) biological process (A) and molecular function (B)
37 categories of silver-regulated proteins in *P. aeruginosa* treated with AgNPs (1.2 $\mu\text{g mL}^{-1}$)
38 and AgNO₃ (0.6 $\mu\text{g mL}^{-1}$).

39 **Experimental methods**

40 **Chemicals**

41 AgNPs with polyvinylpyrrolidone (PVP)-coating (5 nm) was purchased from Huzheng
42 Nanotechnology Co., Ltd. (Shanghai, China). The following proteins were purchased
43 from Sigma-Aldrich (USA): carbonic anhydrase (CA), ovalbumin (OVA), bovine
44 serum albumin (BSA) and conalbumin (CB). Further chemicals used were: iodine,
45 potassium iodate, sodium dithionite (Beijing Chemical Regents Company, China);
46 Tris (Angus Chemical Company, USA). All reagents used for gel preparation were
47 purchased from Merck (Germany). All solutions were prepared using ultrapure water
48 (Milli-Q Advanced A10 system, Millipore, Bedford, USA).

49 **Measurement of silver ion release**

50 As for the determination of silver ion release, the stock solutions of AgNPs were
51 diluted in the sterile deionized water at the concentrations of 0.1, 0.2, 0.4, and 0.6 μg
52 mL^{-1} , respectively, and incubated at 37°C under shaking for 12 and 24 h.
53 Ultrafiltration with 3 kDa cutoff membrane (Millipore) at 9000 g for 10 min was used to
54 collect the dissolved silver ions released from AgNPs. The obtained filtrates were
55 detected by ICP-MS to calculate the amount of silver ion. To validate the feasibility of
56 this method, the recovery efficiency of 0.1 $\mu\text{g mL}^{-1}$ AgNO_3 solution was measured.
57 The recovery of AgNO_3 (as Ag) through ultrafiltration was $93.2 \pm 2.3\%$, suggesting
58 negligible loss of silver ions during the ultrafiltration process. All experiments were
59 performed for three samples.

60 **I-markers synthesis**

61 The protein solutions of CA, OVA, BSA and CB are prepared by diluting protein with
62 0.1 M Tris-buffer (pH 7.5). The reactive iodination reagent KI_3 was generated by
63 saturated 50 mM KI solution with elemental iodine in Tris-buffer (pH 7.5). The
64 solutions of four marker proteins are incubated with KI_3 at a final concentration at 5
65 mM for 5 to 10 min at room temperature. A sodium dithionite solution was added in
66 the reaction as terminator at a final concentration larger than 10 mM. After that, the
67 solutions were ultrafiltered to remove inorganic salts and small molecules by using

68 ultra filtration. All the I-labeled proteins were stored at -80°C before use.

69 **Gel preparation and ICP-MS detection**

70 Native polyacrylamide gels with different concentrations of the monomers were used
71 for the separation of I-markers and proteins. The gel housed in tube contained a
72 varied length resolving gel cast to 15%, 13%, 10%, and 8%, along with a stacking gel
73 of 4%.

74 GE system coupled with ICP-MS via a MicroMist nebulizer and a Scott spray
75 chamber on line. The elution protein is introduced into the nebulizer at the rate of
76 0.14 mL min⁻¹. The operating conditions are listed in Table S4.

77 **Table S1** Differentially expressed proteins identified in *P. aeruginosa* from AgNPs
 78 (1.2 µg mL⁻¹) exposure.

No	Accession	Gene name	Description	Score	Coverage	Fold
1	Q9HT15	atpE	ATP synthase subunit c	92.9	21.18	2.44
2	Q9I0U8	PA2536	Probable phosphatidate cytidyltransferase	38.04	5.14	2.09
3	Q9I022	PA2821	Probable glutathione S-transferase	42.39	7.27	2.01
4	Q9HVR7	PA4504	Probable permease of ABC transporter	25.18	3.3	1.89
5	G3XCX7	pilP	Type 4 fimbrial biogenesis protein PilP	41.37	8.62	1.78
6	Q03268	PA2604	Uncharacterized protein PA2604	139.05	7.21	1.71
7	Q9HWW0	PA4068	Probable epimerase	49.3	8.09	1.71
8	Q9I3G2	CcoP2	Cbb3-type cytochrome c oxidase subunit	47.46	10.26	1.69
9	O52762	KatA	Catalase	26.47	8.46	1.69
10	G3XD11	oprH	PhoP/Q and low Mg ²⁺ inducible outer membrane protein H1	875.56	62.5	1.68
11	O68822	pepA	Cytosol aminopeptidase	623.79	33.54	1.63
12	Q9I787	PA0045	Uncharacterized protein	46.03	6.58	1.62
13	P53641	sodB	Superoxide dismutase [Fe]	667.91	46.63	1.62
14	Q9HVM9	pilX	Type 4 fimbrial biogenesis protein PilX	34.43	7.18	1.61
15	Q9I3G6	PA1551	Probable ferredoxin	60.78	8.07	1.61
16	Q9I0H4	Hmp	Flavo-hemoprotein	23.3	3.45	1.59
17	Q9HWY1	ribA	GTP cyclohydrolase-2	22.13	3.41	1.59
18	Q9HTY0	PA5210	Probable secretion pathway ATPase	64.71	3.37	1.59
19	Q9HZ96	PA3128	Probable short-chain dehydrogenase	27.07	4.03	1.58
20	Q9I3D6	sdhD	Succinate dehydrogenase hydrophobic membrane anchor subunit	60.32	16.39	1.56
21	P50587	pyrE	Orotate phosphoribosyltransferase	282.96	24.41	1.55
22	Q9HWP8	PA4133	Cytochrome c oxidase	31.3	20	1.55
23	Q9HV90	phuT	Heme-transport protein, PhuT	51.52	8.75	1.54
24	Q9HVV8	hisG	ATP phosphoribosyltransferase	32.94	4.27	1.53
25	Q9I669	PA0449	Uncharacterized protein	40.23	6.7	1.52
26	P14532	CcpA	Cytochrome c551 peroxidase	25.61	9.38	1.52
27	P32722	oprD	Porin D	259.33	20.54	1.50
28	Q9HWX3	ribC	Riboflavin synthase alpha chain	61.21	5.94	0.67
29	Q51567	sucD	Succinyl-CoA ligase [ADP-forming] subunit alpha	1526.45	67.8	0.67
30	Q9HZP7	etfA	Electron transfer flavoprotein subunit alpha	1899.42	74.11	0.66
31	Q9I4D6	PA1202	Probable hydrolase	173.83	40	0.66
32	Q9HVQ7	PiuC	PKHD-type hydroxylase PiuC	55.44	9.35	0.66
33	Q9HWB0	PA4290	Probable chemotaxis transducer	359.67	7.62	0.66
34	Q00513	xcpS	Type II secretion system protein F	48	4.69	0.65
35	Q9HZ64	PA3170	Uncharacterized protein	184.45	11.94	0.65
36	Q9HX76	PA3940	Probable DNA binding protein	152.79	35.48	0.65
37	Q51466	fliN	Flagellar motor switch protein FliN	124.7	38.85	0.65
38	Q9I0S7	PA2557	Probable AMP-binding enzyme	55.34	3.55	0.64
39	Q9I793	PA0039	Uncharacterized protein	100.4	32	0.64

40	Q9HUT9	PA4874	Uncharacterized protein	87.48	14.49	0.62
41	Q9HV60	PA4739	Uncharacterized protein	225.99	41.23	0.61
42	Q9HWE6	rplX	50S ribosomal protein L24	284.67	53.85	0.60
43	Q9I4R0	PA1064	Uncharacterized protein	40.07	8.14	0.60
44	P50597	rph	Ribonuclease PH	85.31	7.95	0.59
45	P05384	hupB	DNA-binding protein HU-beta	5078.6	61.11	0.59
46	Q9I3G1	ccoO2	Cytochrome c oxidase, cbb3-type, CcoO subunit	353.81	33.66	0.58
47	Q9HV61	PA4738	UPF0337 protein PA4738	37.21	33.85	0.58
48	Q9I651	PA0467	Uncharacterized protein	92.9	8.74	0.58
49	Q9HZ28	PA3212	Probable ATP-binding component of ABC transporter	118.19	11.74	0.57
50	Q9I544	mgtE	Magnesium transporter MgtE	35.93	3.11	0.55
51	Q9I3G4	ccoO1	Cytochrome c oxidase, cbb3-type, CcoO subunit	168.6	21.18	0.55
52	Q9HUG0	PA5005	Probable carbamoyl transferase	192.73	15.21	0.54
53	Q9I6C2	PA0372	Probable zinc protease	39.1	5.16	0.52
54	G3XD89	oprC	Putative copper transport outer membrane porin OprC	540.22	30.01	0.49
55	Q9I4P9	flgE	Flagellar hook protein FlgE	29.44	7.14	0.49
56	Q9HTW1	PA5229	Uncharacterized protein	42.83	10.53	0.39
57	G3XD39	PA0622	Probable bacteriophage protein	103.14	3.89	0.28
58	Q9I2Y9	PA1748	Probable enoyl-CoA hydratase/isomerase	164.46	24.45	0.24
59	Q9I1K3	PA2270	Probable transcriptional regulator	23.78	7.65	0.24
58	Q9I2Y9	PA1748	Probable enoyl-CoA hydratase/isomerase	164.46	24.45	0.24
59	Q9I1K3	PA2270	Probable transcriptional regulator	23.78	7.65	0.24

80 **Table S2** Differentially expressed proteins identified in *P. aeruginosa* from AgNO₃
 81 (0.6 µg mL⁻¹) exposure.

No	Accession	Gene name	Description	Score	Coverage	Fold
1	Q9I022	PA2821	Probable glutathione S-transferase	42.39	7.27	2.56
2	G3XD11	oprH	PhoP/Q and low Mg ²⁺ inducible outer membrane protein H1	875.56	62.5	2.35
3	Q9I0U8	PA2536	Probable phosphatidate cytidyltransferase	38.04	5.14	1.98
4	Q9HZL7	lolD	Lipoprotein-releasing system ATP-binding protein LolD	32.17	14.54	1.97
5	Q9HT15	atpE	ATP synthase subunit c	92.9	21.18	1.80
7	P42512	fptA	Fe(3+)-pyochelin receptor	223.87	15.97	1.70
8	Q51507	pchB	Isochorismate pyruvate lyase	77.83	33.66	1.65
9	Q9HTY0	PA5210	Probable secretion pathway ATPase	64.71	3.37	1.63
10	O68822	pepA	Cytosol aminopeptidase	623.79	33.54	1.61
11	G3XD66	narX	Histidine kinase	50.32	2.89	1.58
12	Q9I680	codA	Cytosine deaminase	28.05	3.78	1.54
13	Q9HWW1	oprG	Outer membrane protein OprG	7707.45	66.81	1.54
14	P32722	oprD	Porin D	259.33	20.54	1.54
15	Q51508	pchA	Salicylate biosynthesis isochorismate synthase	50.26	3.99	1.51
16	Q9HVD1	pagL	Lipid A deacylase PagL	392.63	48.55	1.50
6	Q9HWP8	PA4133	Cytochrome c oxidase	31.3	20	1.15
17	Q9HUG0	PA5005	Probable carbamoyl transferase	192.73	15.21	0.67
18	Q9HV60	PA4739	Uncharacterized protein	225.99	41.23	0.66
19	Q9HZ64	PA3170	Uncharacterized protein	184.45	11.94	0.66
20	Q9I593	PA0848	Probable alkyl hydroperoxide reductase	28.62	8.54	0.66
21	Q51466	fliN	Flagellar motor switch protein FliN	124.7	38.85	0.66
22	Q9HY08	mutS	DNA mismatch repair protein MutS	65.71	2.34	0.65
23	Q9HTW1	PA5229	Uncharacterized protein	42.83	10.53	0.65
24	Q9HYJ2	PA3413	Uncharacterized protein	27.91	15.91	0.63
25	Q9I4R0	PA1064	Uncharacterized protein	40.07	8.14	0.59
26	Q9I6C2	PA0372	Probable zinc protease	39.1	5.16	0.56
27	G3XD89	oprC	Putative copper transport outer membrane porin OprC	540.22	30.01	0.52
28	O68283	eda	2-dehydro-3-deoxy-phosphogluconate aldolase	122.67	10.91	0.42

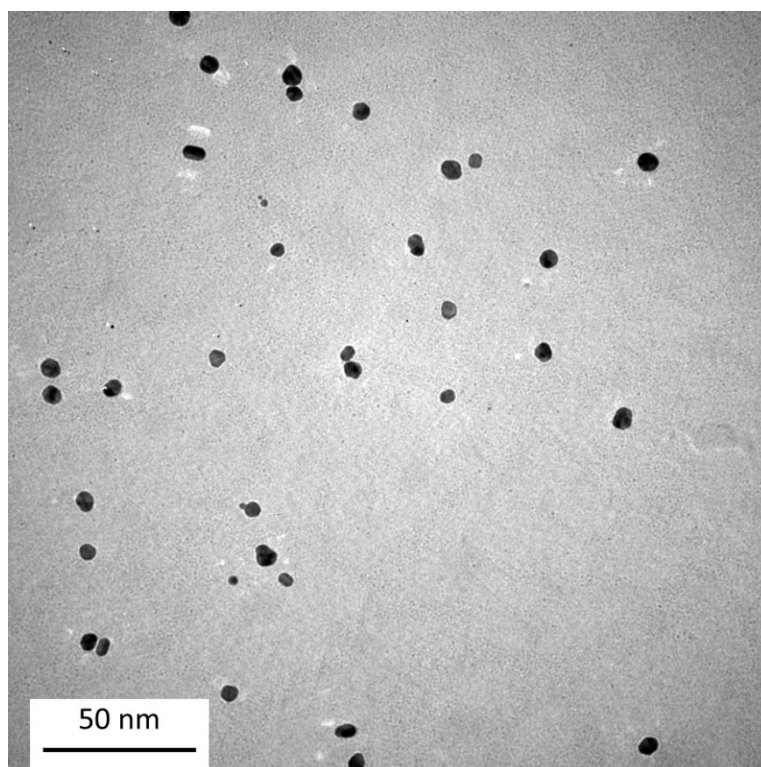
83 **Table S3** Identified silver-binding proteins from *P. aeruginosa*.

No	Accession	Gene name	Description	Protein score	Molecular weight (Daltons)
1	P30718	GroL	60 kDa chaperonin	29132.13	57086
2	P09591	TufA	Elongation factor Tu	32319.69	43370
3	Q83WT8	FliC	Flagellin	545.88	39431
4	Q9HZP7	EtfA	Electron transfer flavoprotein subunit alpha	8025	31423
5	PA3309	PA3309	Uncharacterized protein	7309	16496

85 **Table S4.** ICP-MS operating conditions

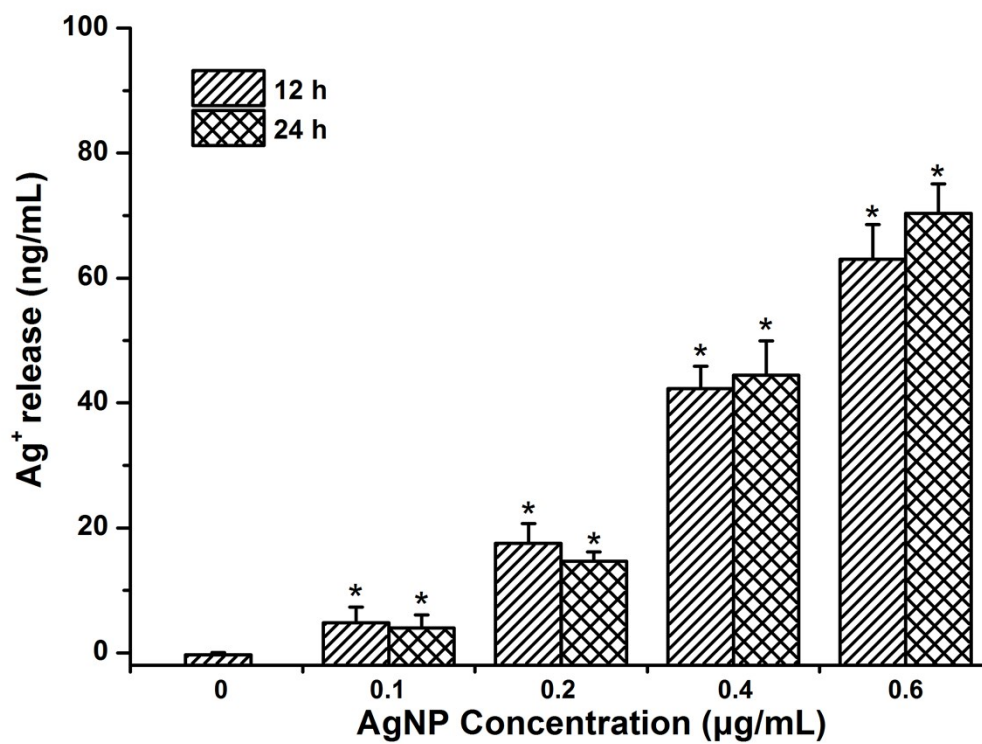
parameters	Setting
Sampler cones	Ni, 1.0 mm orifice
Skimmer cones	Ni, 0.7 mm orifice
RF power	1300 W
Coolant gas flow rate	15.0 L min⁻¹
Nebuliser gas flow rate	0.8 L min⁻¹
Auxiliary gas flow rate	0.8 L min⁻¹
Dwell time	100 ms

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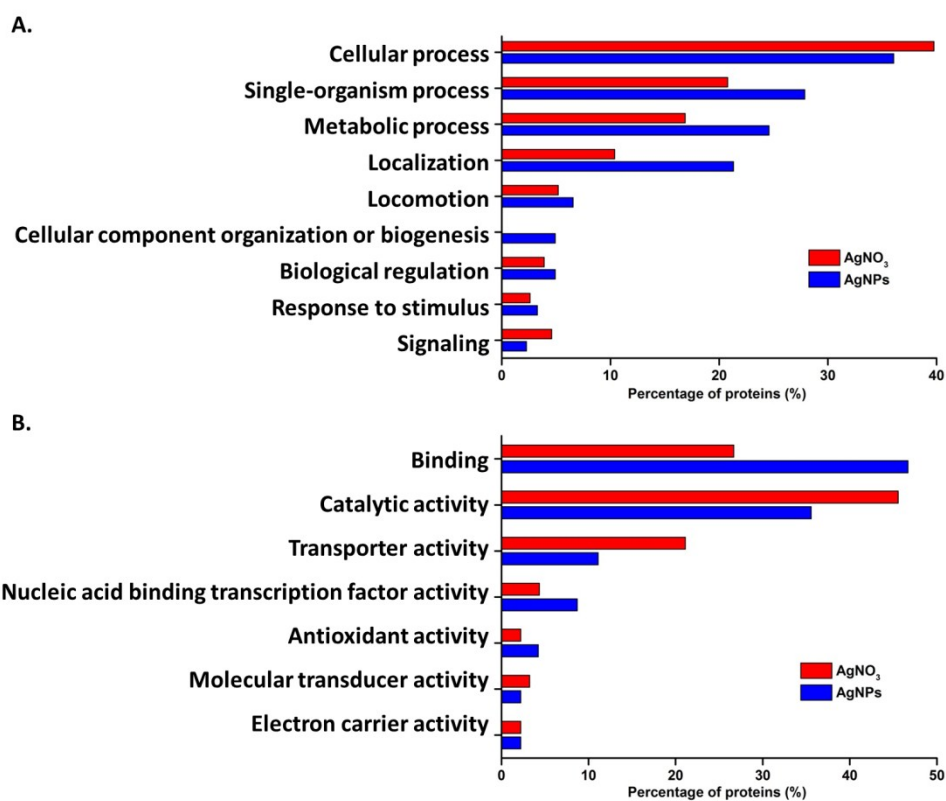
88 **Fig. S1** TEM image of 5 nm AgNPs. An aliquot of sonicated AgNPs suspension was
89 dispersed on the carbon membrane coated copper net and dried at room
90 temperature.



91

92 **Fig. S2** Release of silver ions from AgNPs after 12 h and 24 h. The initial
93 concentrations of total silver content were 0.1, 0.2, 0.4, and 0.6 µg mL⁻¹, respectively.

94 Significantly different from control: * $p < 0.05$.



95

96 **Fig. S3** Major Gene ontology (GO) biological process (A) and molecular function (B)
 97 categories of silver-regulated proteins in *P. aeruginosa* treated with AgNPs (1.2 μg
 98 mL^{-1}) and AgNO₃ (0.6 μg mL^{-1}).