

Supporting Information

Biosynthesis of Au, Ag and Au-Ag bimetallic nanoparticles using protein extracts of *Deinococcus radiodurans* and evaluation of their cytotoxicity

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Methods

Identification of proteins on the surface of AuNPs synthesized by *D. radiodurans*

The prepared gold nanoparticles using *D. radiodurans* were repeatedly washed using ultrapure water, then boiled with 1% SDS solution for 10 min. After centrifugation at 8000 × *g* for 10 min, the supernatant was collected and condensed. The samples were further analyzed by 12% SDS-PAGE as previously reported.^{1,2} The significant gel slice was collected and the proteins in it was identified by MS (Triple TOF 5600 + LC/MS/MS system, AB SCIEX, USA).³

The identification of proteins was performed according to the *D. radiodurans* protein sequence database (NCBI). The unweighted spectrum count of each protein was divided using its mass, and was used as the indicator of relative protein abundance in each sample.⁴

Figures

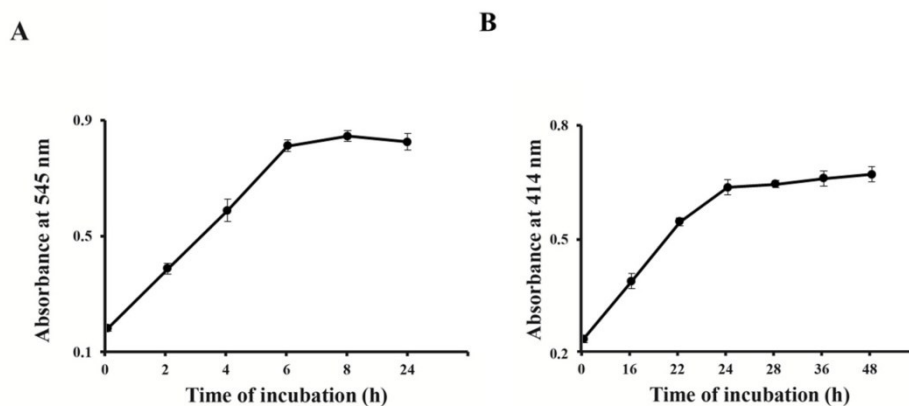


Figure S1 UV-visible kinetic study of nanoparticles.

Note: (A) The absorbance at 545 nm for the Drp-AuNPs formation during the incubation time of 24 h at room temperature and pH 7. (B) The absorbance at 414 nm for the Drp-AgNPs formation during the incubation time of 48 h at room temperature and pH 7.

Abbreviation: Drp-AuNPs, *D. radiodurans* protein extract-mediated gold nanoparticles; Drp-AgNPs, *D. radiodurans* protein extract-mediated silver nanoparticles.

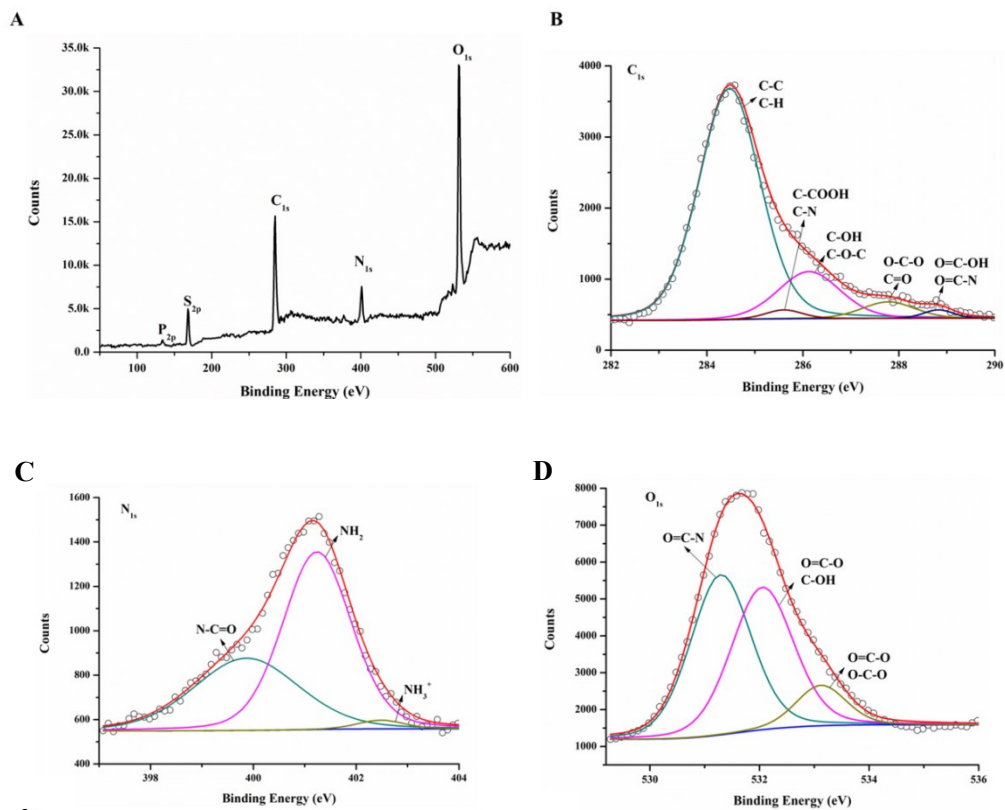


Figure S2 XPS analysis of protein extracts from *D. radiodurans* **(A)** and the core level C_{1s} **(B)**, N_{1s} **(C)** and O_{1s} **(D)** of the protein extracts.

Note: **(A)** The wide scan spectrum of proteins included spectrum of O_{1s} , N_{1s} , C_{1s} , S_{2p} and P_{2p} , **(B)** The peak at 284.6 eV could be attributed to carbon bonded with carbon (C–C) and hydrogen (C–H). Carbon bound to nitrogen (C–N) and hydroxyl groups (C–OH), for amine, amide, and alcohol groups had binding energies at 285.7 and 286.5 eV, respectively. **(C)** The peak at 287.9 eV was assigned to carbonyl groups (aldehydic or ketonic carbon). The high binding energy peak at 289.2 eV was ascribed to amide carbon (N–C=O) and carboxylate (O=C–OH) of proteins. The N_{1s} core level centred at 399.6 and 401.3 eV were assigned to unprotonated amine and amide groups respectively, whereas the high binding energy peak at 402.5 eV suggested protonation of amino groups. **(D)** Three distinct peaks at 531.2, 532.1 and 533.2 eV in the O_{1s} spectrum corresponded to C=O, C–OH and O–C–O components of the

protein extracts, respectively.

Abbreviations: XPS, X-ray photoelectron spectroscopy.

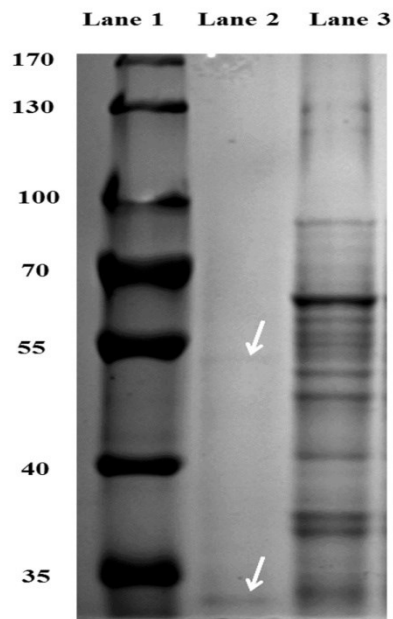


Figure S3 SDS-PAGE analysis of protein profile bound to AuNPs synthesized by *D. radiodurans* cells.

Note: Lane 1, molecular size marker. Lane 2, the protein profile bound to AuNPs synthesized by *D. radiodurans* cells. The arrows in Lane 2 indicate two protein bands of ~55 and ~35 kDa, confirming the presence of proteins on the surface of AuNPs synthesized by *D. radiodurans*.

Lane 3, proteins extracted from *D. radiodurans*.

Abbreviations: SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

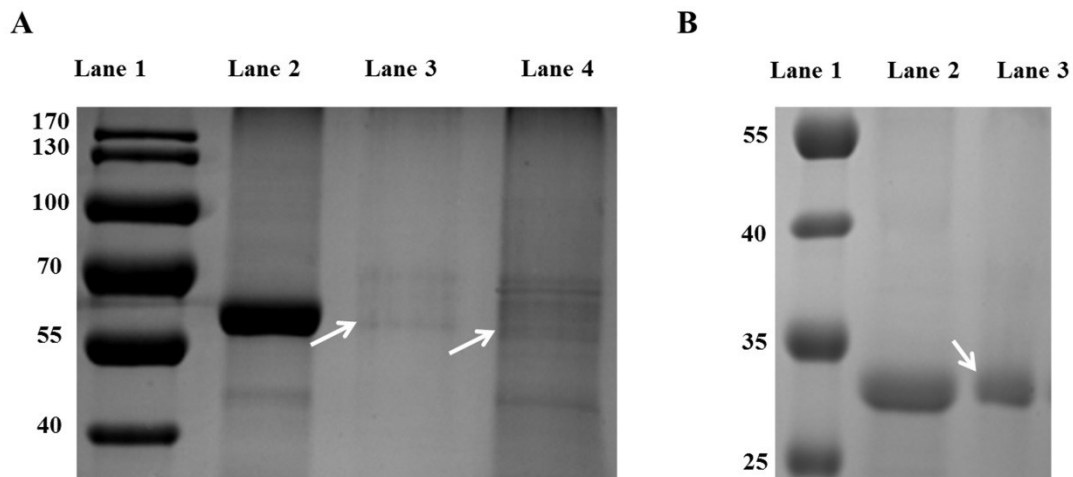


Figure S4 SDS-PAGE analyses of purified proteins of CrtI (**A**) and Dps2 (**B**) as reductant and capping agents in the syntheses of AuNPs and AgNPs.

Note: (**A**), Lane 1, molecular size marker; Lane 2, the purified CrtI; Lane 3 and Lane 4, the protein profile bound to AuNPs and AgNPs, respectively; (**B**), Lane 1, molecular size marker; Lane 2, purified Dps2; Lane 3, the protein profile bound to AgNPs. The arrows indicated the proteins separated from the nanoparticles.

Abbreviation: SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; AuNPs, gold nanoparticles; AgNPs, silver nanoparticles.

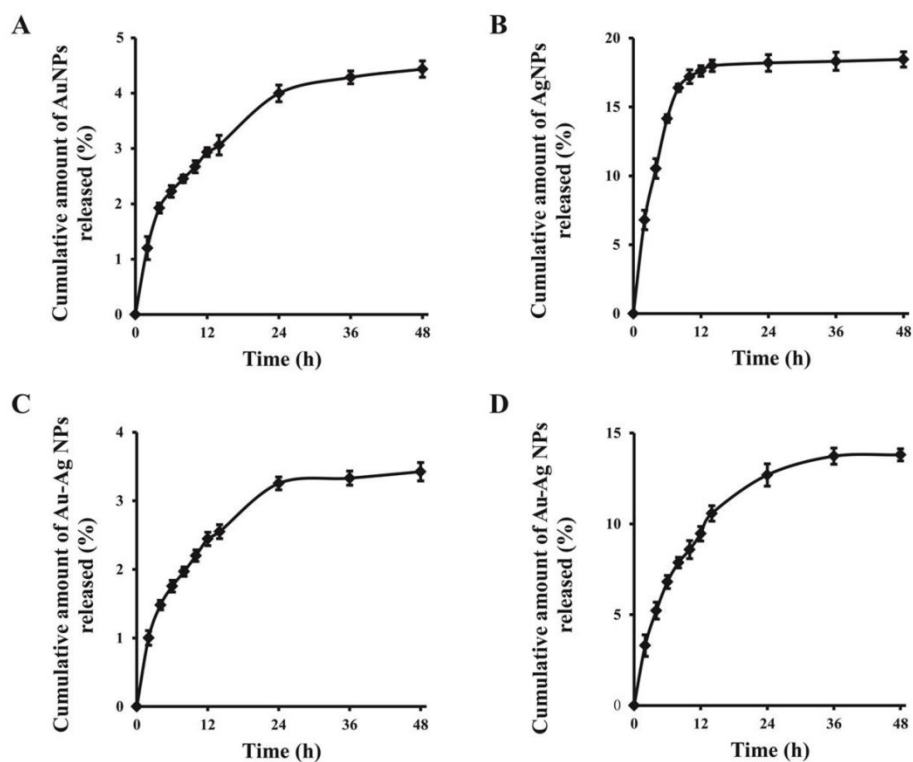


Figure S5 Curves for metal release kinetics of Drp-AuNPs (**A**), Drp-AgNPs (**B**), Au release kinetics of Au–Ag bimetallic NPs (**C**), Ag release kinetics of Au–Ag bimetallic NPs (**D**), over a period of 48 h.

Abbreviations: Drp-AuNPs, *D. radiodurans* protein extract-mediated gold nanoparticles; Drp-AgNPs, *D. radiodurans* protein extract-mediated silver nanoparticles; NPs, nanoparticles.

Table S1: Identified proteins from the surface of AuNPs synthesized by *D. radiodurans* cultures.

Number	Protein MW (Da)	Protein Name	Relative Abundance Index
1	123,730.9	S-layer protein	+ + +
2	65,621.5	peptide ABC transporter periplasmic peptide-binding protein	+ +
3	42,119.5	yellow-like protein	+
4	33,085.7	hypothetical protein DR_1481	+ +
5	33,447.8	immunogenic protein	+ +
6	76,755.6	serine protease	+
7	36,707.7	S-layer-like array-like protein	+ +
8	26,977.4	50S ribosomal protein L25/general stress protein Ctc	+
9	33,972.1	ABC transporter periplasmic substrate-binding protein	+ +
10	47,281.1	carboxyl-terminal protease	+ +
11	57,534	extracellular solute-binding protein	+
12	37,221.2	peptide ABC transporter permease	+ +
13	31,687.6	3-hydroxybutyryl-CoA dehydratase pyruvate dehydrogenase complex, dihydrolipoamide acetyltransferase E2 component	+

14	63,661	electron transfer flavoprotein subunit alpha	+
15	32,756.2	electron transfer flavoprotein subunit beta	++
16	27,259.1	metal binding protein	++
17	37,977.3	ABC transporter periplasmic amino acid-binding protein	++
18	28,054.4	formyltetrahydrofolate deformylase	+
19	33,388.6	fructose-bisphosphate aldolase	+
20	32,421.3	histidinol-phosphate aminotransferase	+
21	39,458.4	hypothetical protein DR_1448	++
22	45,867	hypothetical protein DR_2310	++
23	84,410.5	peptide ABC transporter periplasmic peptide-binding protein	+
24	64,842.8	putative manganese-dependent inorganic pyrophosphatase	+
25	34,039.1	thiosulfate sulfurtransferase	++
26	41,287.9	NADH dehydrogenase II	+

Note: MS analysis of the proteins in the ~35 kDa band separated by SDS-PAGE (Figure S3). Relative protein abundance was indicated by the index value (i = unweighted spectrum count of each protein/mass). +, $i \leq 0.20$; ++, $0.20 < i \leq 0.40$; +++, $0.40 < i \leq 0.60$; + + + +, $0.60 < i \leq 0.80$; + + + + +, $i \geq 0.80$. A total of 26 convective proteins including S-layer protein were identified on the AuNPs' surface.

Abbreviations: AuNPs, gold nanoparticles.

Table S2: Amino acid compositions of Crtl and Dps2 from *D. radiodurans*.

Name	Total number of amino acids	Number of amino acid of tryptophan (W)	Number of amino acid of tyrosine (Y)
Crtl	548	3	22
Dps2	241	3	6

Sequence of Crtl:

MTSALPRPAPSPYARRKTALVIGAGFGGLALGIRLQSLGFDTTILERLDGPGGRAYQKRTPDG
YVFDMGPTVLTVPHFIEELFALERDRAGLDAPDY PPEVL SGERVKEGVSGGPHTSRVYTLVPI
LPFYRIVFHDGT YFDYDGDPESTRRQIAELAPGDLAGYERFHADAEAFRRGFLELG YTHFGD
VPTMLRVVPDLLKLDVRTLFSFTSKYFQSDKLRQVFSFETLLVGGNPLSVPAYAMIHFVEKT
WGIHYAMGGTGALVRGLVQKFEELGGAIRY GAGVDEVLDGNLPGKRTARGVRLESGEELR
ADLVASNGDWANTY LKRV RPSARLVNSDLRVKAASESMSLLVYFGFRGGDDLPLKHHNILL
GPRYEALLSEIFGTKRLGEDFSQYLHVPTLTDPALAPAGHHAAYTLVPVPHNGSGIDW DVEGP
KLAEAAADIERRGLIPGLRERLTHFEFITPDYFAGTLD SYLGNAFGPEPRLVQSAFFRPHNRS
EDLHNFYLVGAGAQP GAGTPSVMM SAKMTARLIAEDFGIHADIRR.

Sequence of Dps2:

MRHSVKTVVVVSSLLLGTALAGGAGAQSAGNGVPSTNVNTPAPNTGQSTAQNTNTASPLPY
NRATTLPAAGTEDLKKS VQALQNTLTELQALQLQTKQAHWNVSGTLWYTLHELLQDHYEGIS
KFADDVAERQLSVGASSDGRAITIVAASRLPEIPGGFLDDAQVIQFFTYYETVGQRIHQRVGD
VEKVDPTTANLLQEVEHIIEKYQWQMRAFLQNTPTDPNTGFDINNGKPVPLRGR.

Note: Tyrosine was represented as the red capital of Y and tryptophan was represented as the green capital of W.

References

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4. Estrada ML, Fan E, Odermatt M, et al. TtOmp85, a β -Barrel Assembly Protein, Functions by Barrel Augmentation. *Biochemistry*. 2015;54(3):844-852.