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Supporting information

Efficient bioremediation of radioactive iodine Q1 Q2 using biogenic gold nanomaterial-containing radiation-resistant bacterium, Deinococcus radiodurans R1†

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Experimental section

1. Removal of radioactive iodines using gold nanoparticles

Citrate stabilized gold nanoparticles (AuNPs) with diffrent sizes (13 nm, 30 nm, 50 nm, and 80 nm) were added to [^{125}I]NaI solution. The molar ratio of [^{125}I]NaI to AuNPs was 100. After 15 min incubation at room temperaure, the efficiency of each desalination experiment was determined by using radio-TLC analysis (see the equation (i) and figure S7).

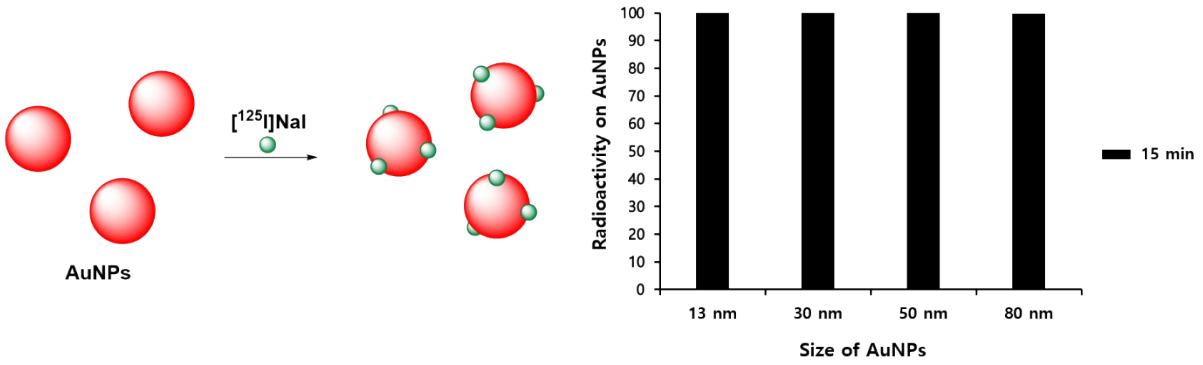

Figure S1. Adsorption of [^{125}I]NaI on various size of AuNPs.

To determine the sustainability of the removal system, 10 μL of NaCl (1.5 M) or DTT solution (1.5 M) was added to 90 μL of ^{125}I-captured 13 nm gold nanoparticles at room temperature. The amount of released radioactivity from gold nanoparticles was then analysed by radio-TLC for 6 h (see the equation (i) and figure S7).

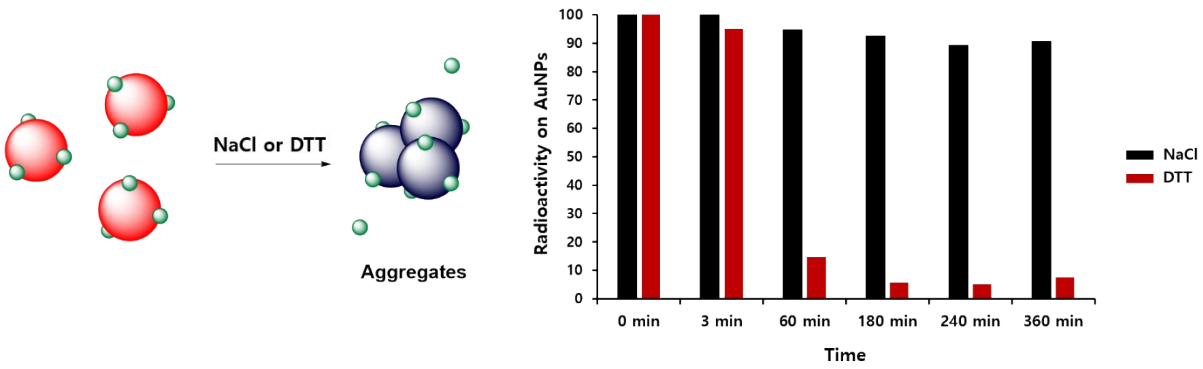

Figure S2. Desorption of [^{125}I]NaI from AuNPs by adding NaCl or DTT

2. Bacterial cultivation

Deinococcus radiodurans R1 (ATCC 13939, DR), a radiation-resistant bacterium used in this study, was grown in TGY medium containing 0.5% tryptone, 0.1% glucose, and 0.3% yeast extract. *D. radiodurans* R1 cells were inoculated in 3 mL TGY broth and incubated at 30 ℃ with agitation (200 rpm) for 24 h. 500 μL of pre-cultured cells were transferred to 50 mL fresh TGY broth and incubated until OD value at 1.0.

3. *In vivo* synthesis of gold nanomaterials by *D. radiodurans* R1

Chloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$) was purchased from Sigma-Aldrich Korea. $HAuCl_4 \cdot 3H_2O$ stock solution (125 mM, 0.5 mL) was added to TGY broth with or without the DR cells (OD 1.0, 50 mL) as the final concentration of 1.25 mM and incubated for 16 h at 30 ℃ with agitation (200 rpm). The color of cell broth was changed from orange-pink to dark blue (Figure S3). After cultivation with gold salts for 16 h, cells were harvested by centrifugation at 4,000 rpm for 30 min at 4 ºC and washed three times with deionized water. UV absorption spectrum of *D. radiodurans* R1 and gold nanomaterials-embeded *D. radiodurans* R1 (Au-DR) were measured by using UV-vis spectrophotometer (SpectraMax M3, Molecular Devices). The absorbance of cell solutions was measured from 450 nm to 700 nm on UV/vis spectrophotometer. UV spectrum of Au-DR showed a broad absorption (500-600 nm) and the maximum wavelength of Au-DR was observed at 551 nm (Figure S4).

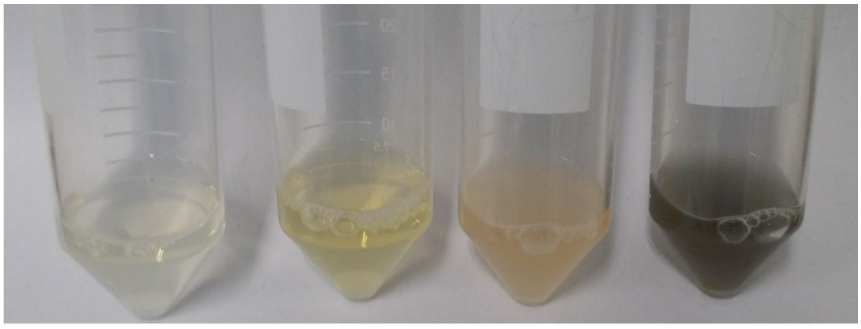

Figure S3. Biosynthesis of AuNP by DR in TGY liquid media.

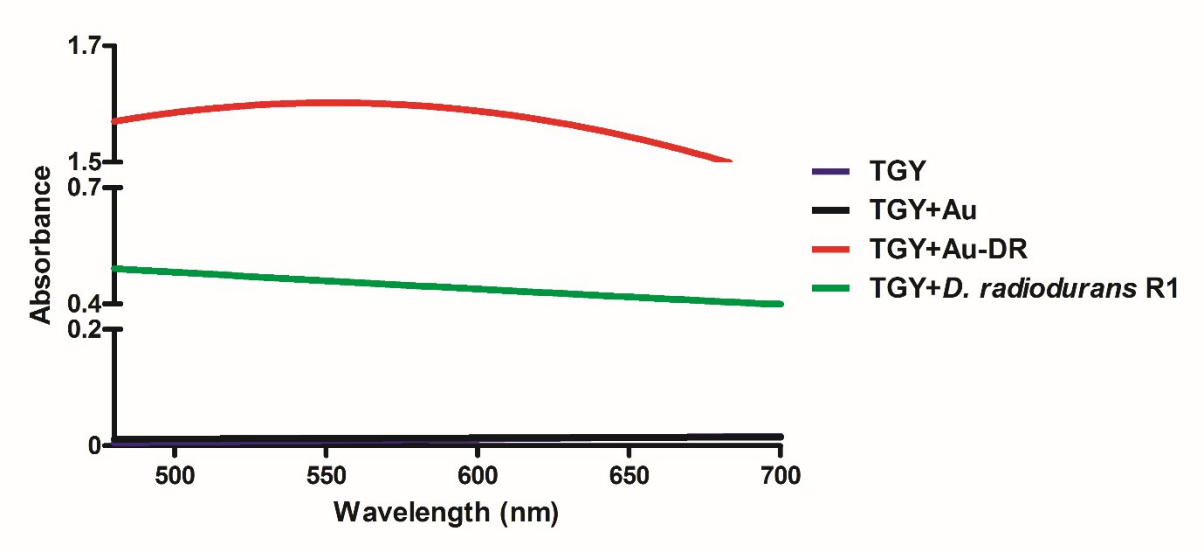

Figure S4. UV-vis spectrum of Au-DR and *D. radiodurans* R1.

3. Characterization of biogenic gold nanomaterials.

Field emission scanning electron microscopy (FE-SEM) analysis. Au-DR cells were harvested and washed three times with deionized water. The cell suspensions were disrupted by bead-beating using FastPrep®-24 Instrument (MP Biomedicals, Korea) at 6 m/s for 1 min and filtered using 0.22 μm syringe filters to purify gold nanomaterials from disrupted samples. Purified samples were dropped on carbon tape and air-dried for 10 min. Then the samples were coated with platinium using ion sputter. The morphology of gold nanomaterials was observed using FEI Verios 460L field emission scanning electron microscope (FEI, Oregon, USA) under the high performance conditions with accelerating voltages up to 15 kV. Elemental composition of gold nanomaterial was analyzed by SEM-energy dispersive X-ray (EDX) (AMITEC) analysis with accelerating voltages up to 20 kV. EDX spectrum was recorded in the area scan mode by focusing the electron beam onto a region of the sample surface (Figure S5).

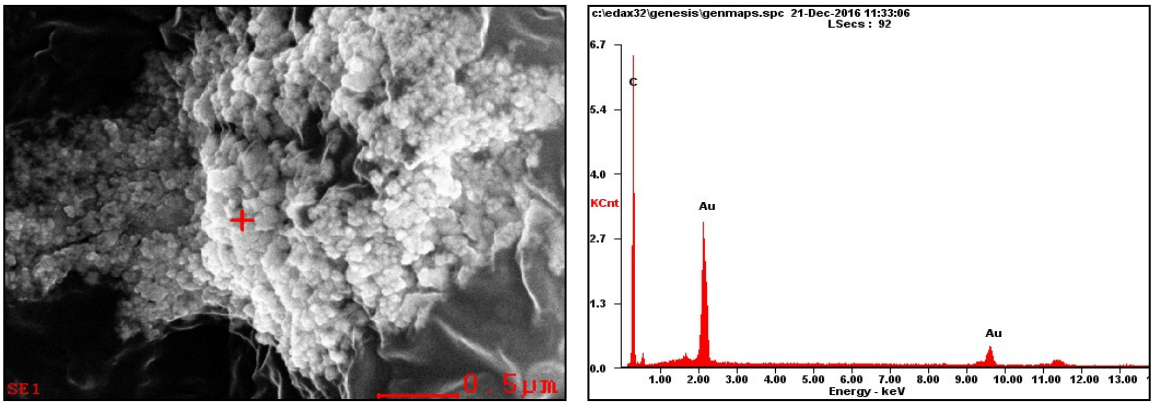

Figure S5. EDX analysis of biogenic gold nanomaterials by *D. radiodurans* R1.

DLS analysis. Au-DR cells were disrupted and centrifuged to separate cell debris and biogenic gold nanomaterial. The supernatants were taken and 10-fold diluted in nuclease-free water. Size distribution of biogenic gold nanomaterial was determined by a Malvern Zetasizer Nano-ZS90 and the result was analyzed by Zetasizer software (Malvern Instruments). All measurements were carried out at 25 °C and three measurements with at least 10 sub-runs were performed for each sample.

TEM analysis. For TEM imaging study, Au-DR cells were placed on carbon-coated copper grides (Gilder Grids) and all samples were observed with accelerating voltages up to 120 kV (Hitachi, H-7650).

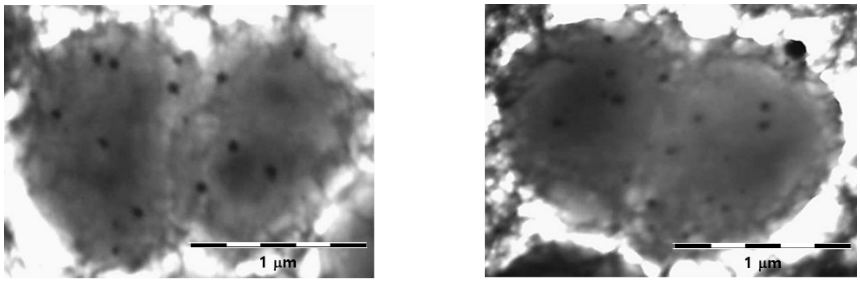

Figure S6. TEM images of Au-DR

Dark-field microscopy analysis. Dark-field imaging of Au-DR was carried out by using Olympus BX-43 (Olympus Corporation, Tokyo, Japan) microscope, equipped with an oil (or water) immersion dark-field condenser and CCD camera. A objective lens (60×) was used for cell imaging.

4. Remediation procedure of radioactive iodine ([^{125}I]NaI) using Au-DR.

[^{125}I]NaI solution (5 μL, 3.7 MBq) (New Korea Industrial Co. Ltd) was added to Au-DR suspended in aqueous media (water, PBS, and synthetic urine). The mixture was shaken on an orbital shaker for 60 min. At each time points (15 sec, 1 min, 5 min, 15 min, 30 min, and 60 min), an aliquot (0.2 μL) was withdrawn from Au-DR solution and it was applied onto a silica-coated thin layer chromatography (TLC) plate. The TLC plate was then developed using acetone as a mobile phase. After the solvent traveled to the top of the plate, the TLC plate was placed on a radio-TLC scanner (Biosacn, AR-2000) and the removal efficiency was monitored by using the scanner and a radioactivity dose calibrator (Capintec, Inc). The captured radioactivity by the Au-DR (or gold nanomaterials) was detected at the bottom position. But free [^{125}I]NaI was eluted to higher position (R_f = 0.8) on TLC (Figure S7).

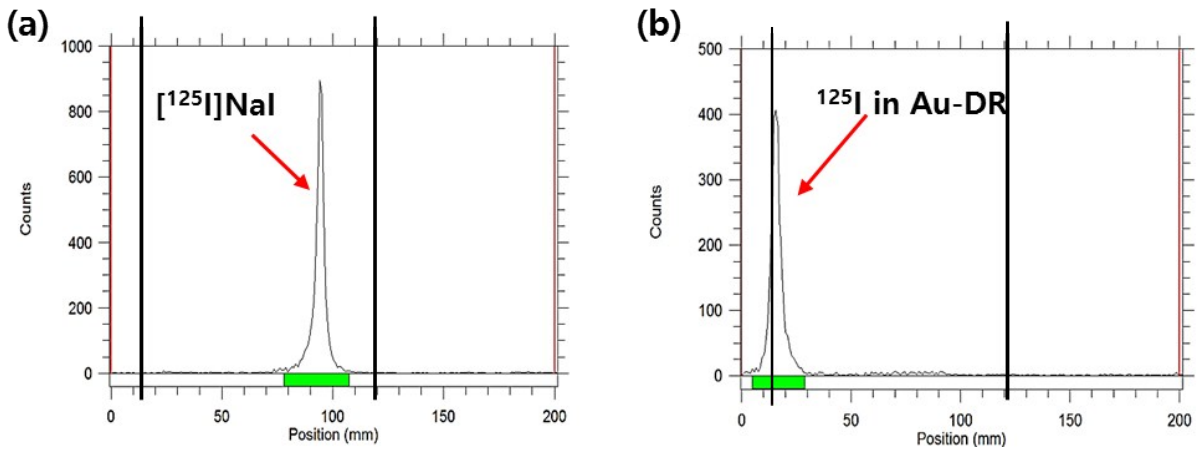

Figure S7. Radio-TLC results (a) free iodine ([^{125}I]NaI) in solution and (b) ^{125}I captured by Au-DR (30 min after the remediation procedure)

The removal efficiency (%) was defined by the following equation to assess the removal capability of Au-DR towards radioactive iodine:

$$\text{Removal efficiency (\%)} = A_1/(A_1 + A_2) \times 100 \quad \text{(i)}$$

where A_1 and A_2 represent the amount of radioactivity at the R_f value of 0 and 0.8 on radio-TLC respectively.

In the remediation experiment (Figure 3a, 1 x PBS), distribution coefficient (K_d) was determined by the following equation:

$$K_d = (C_0 - C_e)/C_e \times \frac{V}{M} \quad \text{(ii)}$$

where C_0 and C_e represent the initial concentration of ^{125}I and the equilibrium concentration in aqueous solution respectively. V denotes the volume of ^{125}I solution (5 mL) and M is the mass of gold nanomaterial in the cell. Assume that all chloroauric acid was converted to nanomaterial, the mass of gold nanomaterial in *D. radiodurans* can be calculated as follows.
Mass of gold nanomaterial in *D. radiodurans* = 125 mM x 0.5 mL x 196.97 (g/mol) = 12.3 mg
As the removal efficiency was observed to be 99.9%, K_d value of the remediation experiment (Figure 3a, 1 x PBS) was determined to be 4.06 x 10^5 mL/g.

To evauate the reusability of Au-DR, the same bioremediation procedure described above (3.7 MBq of [^{125}I]NaI) was repeated for seven times at 1 h intervals. Removal efficiency was determied by using radio-TLC analysis (Figure S8).

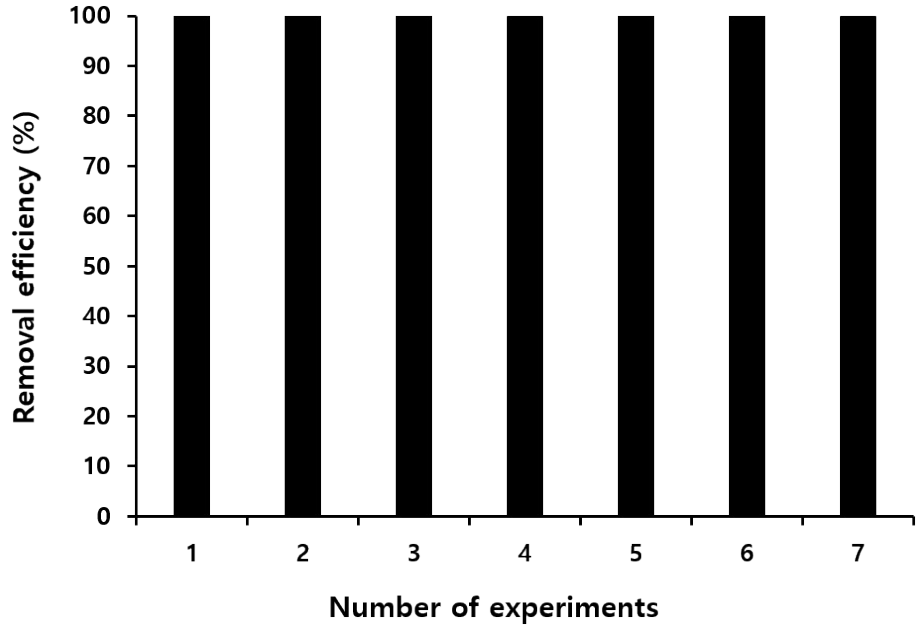

Figure S8. Reusability test of Au-DR

To investigate desorption rate of radioactivy from the cell, three ^{125}I (3.7 MBq) captured Au-DR suspended in pure water, 1 x PBS, and synthetic urine (1 mL for each cell) were prepared. Residual radioactivity in aqueous solutions were monitored using radio-TLC analysis for 24 h (Figure S9). Amount of radioactivity retained in the cell was calcualted by the equation (i).

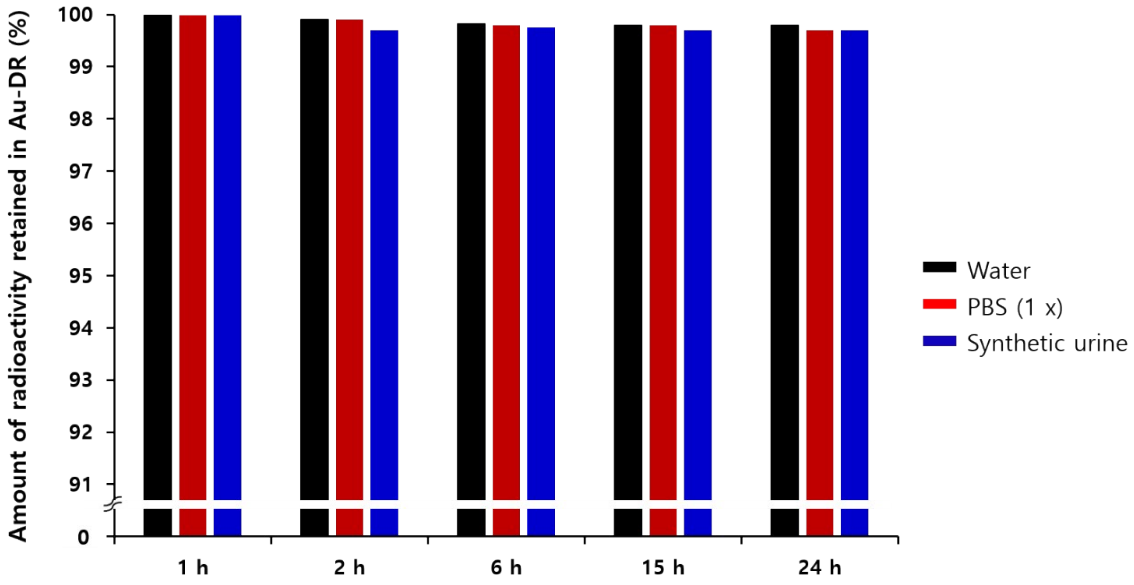

Figure S9. Desorption of radioactive iodine from Au-DR

5. SPECT/CT Imaging of AuNP in *D. radiodurans*. To both Au-DR and *D. radiodurans* R1 cells suspended in 5 mL of pure water, 5 μL of [^{125}I]NaI (3.7 MBq) was added and then the resulting mixture was shaken on a rotary shaker for 30 min. Next, a part of each cells was transferred to 1.5 mL tube and it was subjected to centrifugation for spinning down of the cells (5000 rpm for 5 min). SPECT/CT images of each tube were obtained by using Inveon SPECT/CT system (Siemens, USA).

6. Cell viability exposed to gamma radiation. *D. radiodurans* and Au-DR were taken at 16 h and washed three times before cell viability assay. *D. radiodurans* and Au-DR were exposed to different doses of gamma radiation (2, 4, 6, and 8 kGy) using the cobalt-60 gamma irradiator (point source; AECL, IR-79). After radiation, serially diluted *D. radiodurans* were spotted onto TGY agar plate) and incubated at 30 °C for 3 days. Survived cells were normalized by control cells (0 kGy).

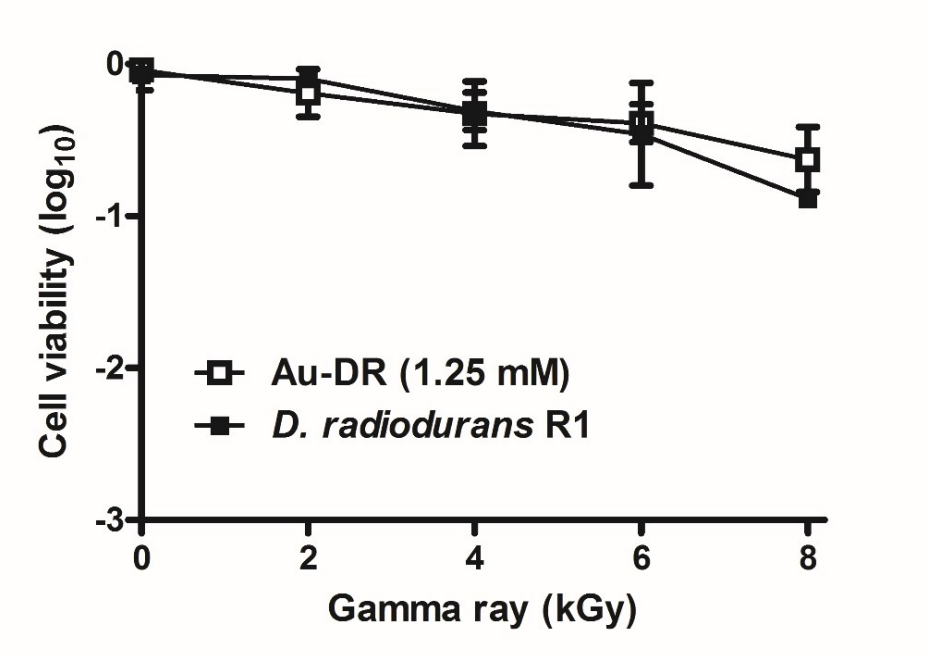

Figure S10. The measurement of the cell viability of *D. radiodurans* (a) and Au-DR (b) exposed to gamma radiation.

Table S1. Comparison to known desalination methods

Adsorbent	Contact time (h)	Removal efficiency (%)	Reference in manuscript
Cu(I) sulfide minerals	>24	>70	16
Cupric metal complex	>100	High	15
Zeolites	2 (at 550 °C)	>99	21
Activated carbons	0.5	70	19
Metal-oraganic framworks	48	>95	24
Metal-oraganic framworks	>24	>90	22
Deep eutectic solvent	5	>99	23
Microporous polymers	48	56	25
Silver oxide titanate nanomaterials	<0.5	>99	17