Supplemental Information

Imposed Environmental Stresses Facilitate Cell-free Nanoparticle Formation by *Deinococcus radiodurans*

Angela Chen,[a] Lydia M. Contreras,*[a] Benjamin K. Keitz *[a]

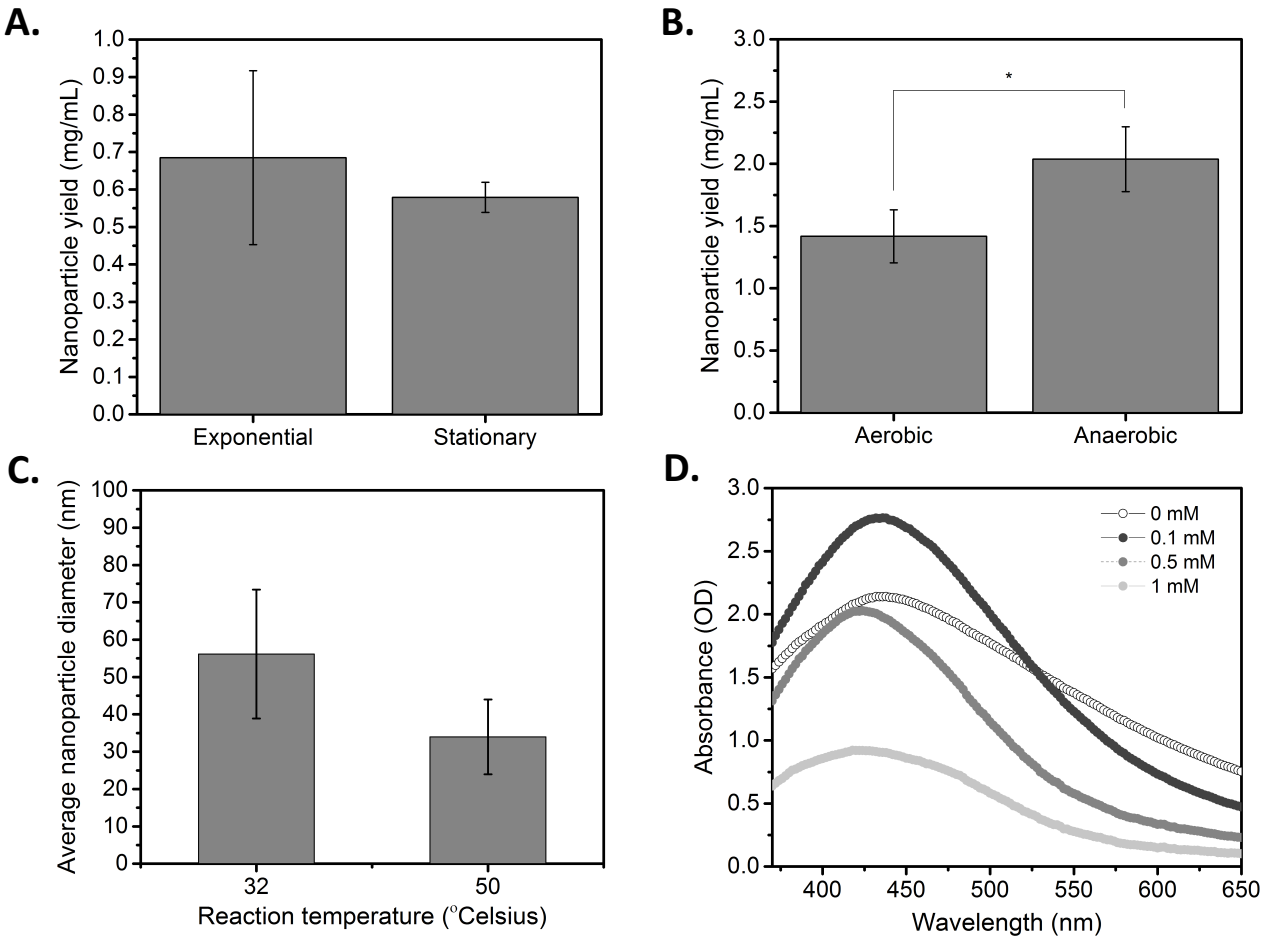

Figure S1 Effect of culturing and reaction conditions on silver nanoparticle yields. (A) Yield data for silver nanoparticle production using exponential and stationary phase supernatant after normalization by OD_{600}. (B) Yield data for silver nanoparticle production using stationary phase supernatant under aerobic and anaerobic conditions (* = $p < 0.05$). (C) Size distribution of silver nanoparticles produced with a reaction temperature of 32 ºC or 50 ºC. (D) UV/vis spectra of silver nanoparticles produced cell-free with different concentrations of silver stressor. Data represents mean ± standard deviation of three replicates.

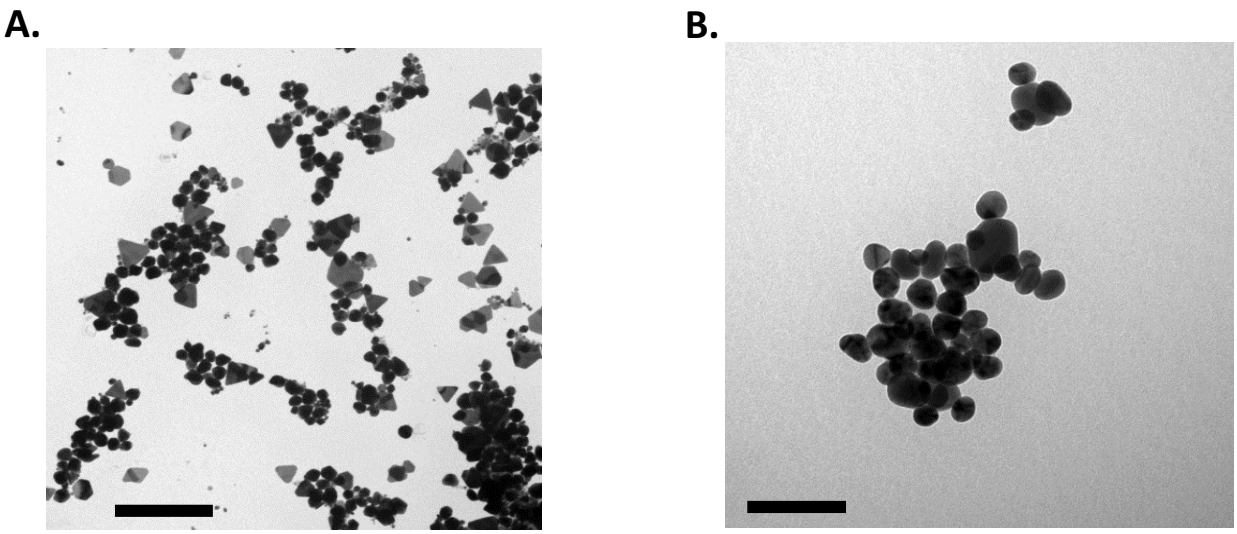

Figure S2 TEM characterization of silver nanoparticles. (A) Bright-field TEM image of silver nanoparticles produced in the absence of an environmental stressor at 32 °C. (B) Bright-field TEM image of silver nanoparticles produced with 1 mM $AgNO_3$ as a stressor at 32 °C. Scale bar in both images is 100 nm.

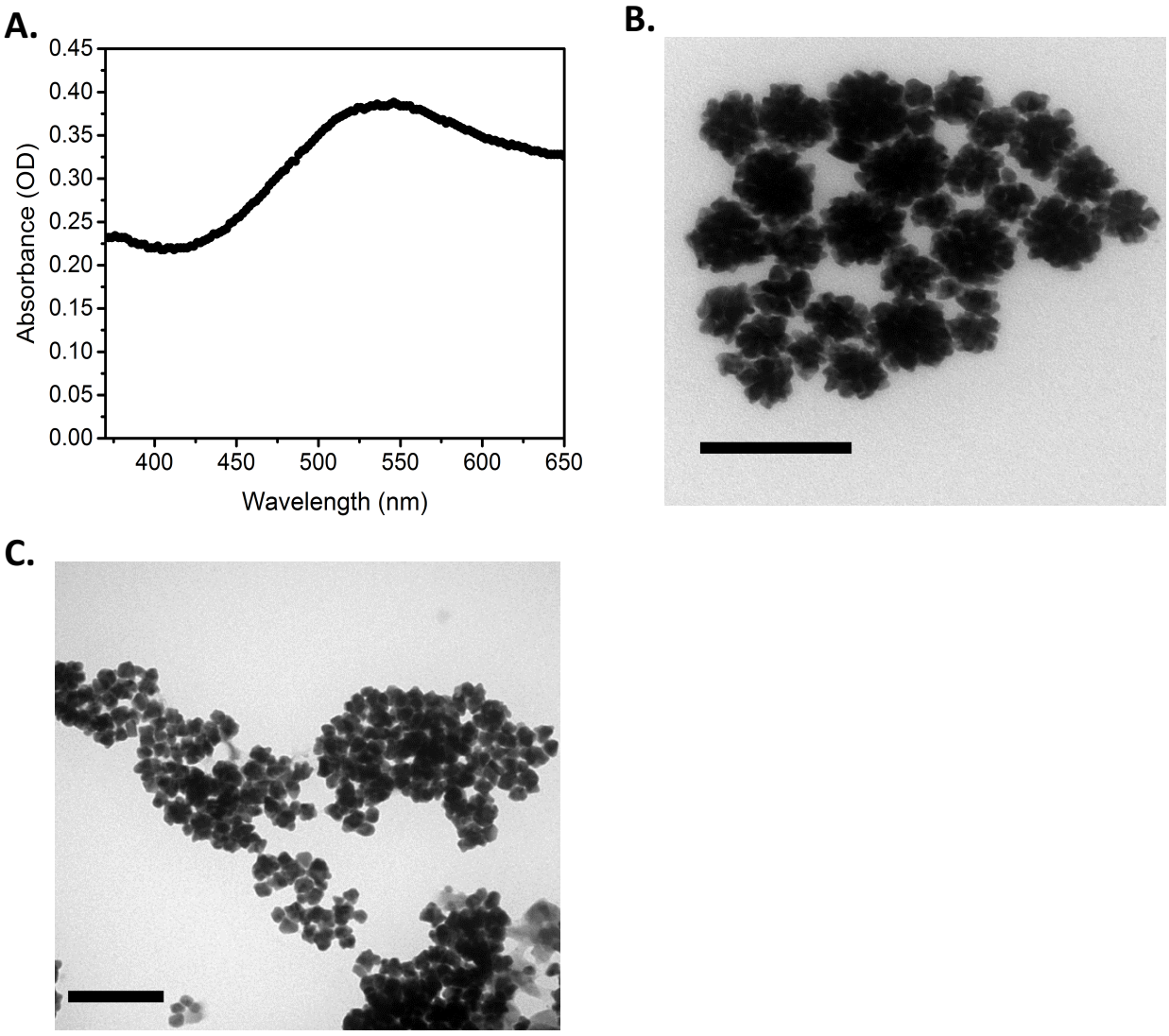

Figure S3 Characterization of bimetallic Ag-AuNPs. (A) UV/vis absorption spectra for Ag-AuNPs produced using 0.5 mM $AgNO_3$ as a stressor and 1 mM $HAuCl_4$ as a metal target. A characteristic peak near 546 nm indicates presence of gold. (B) Bright-field TEM image of Ag-AuNPs produced using 0.5 mM $AgNO_3$ as a stressor and 1 mM $HAuCl_4$ as a metal target. (C) Bright-field TEM image of Ag-AuNPs produced using 1 mM $AgNO_3$ as a stressor and 1 mM $HAuCl_4$ as a metal target. Both scale bars are 100 nm.

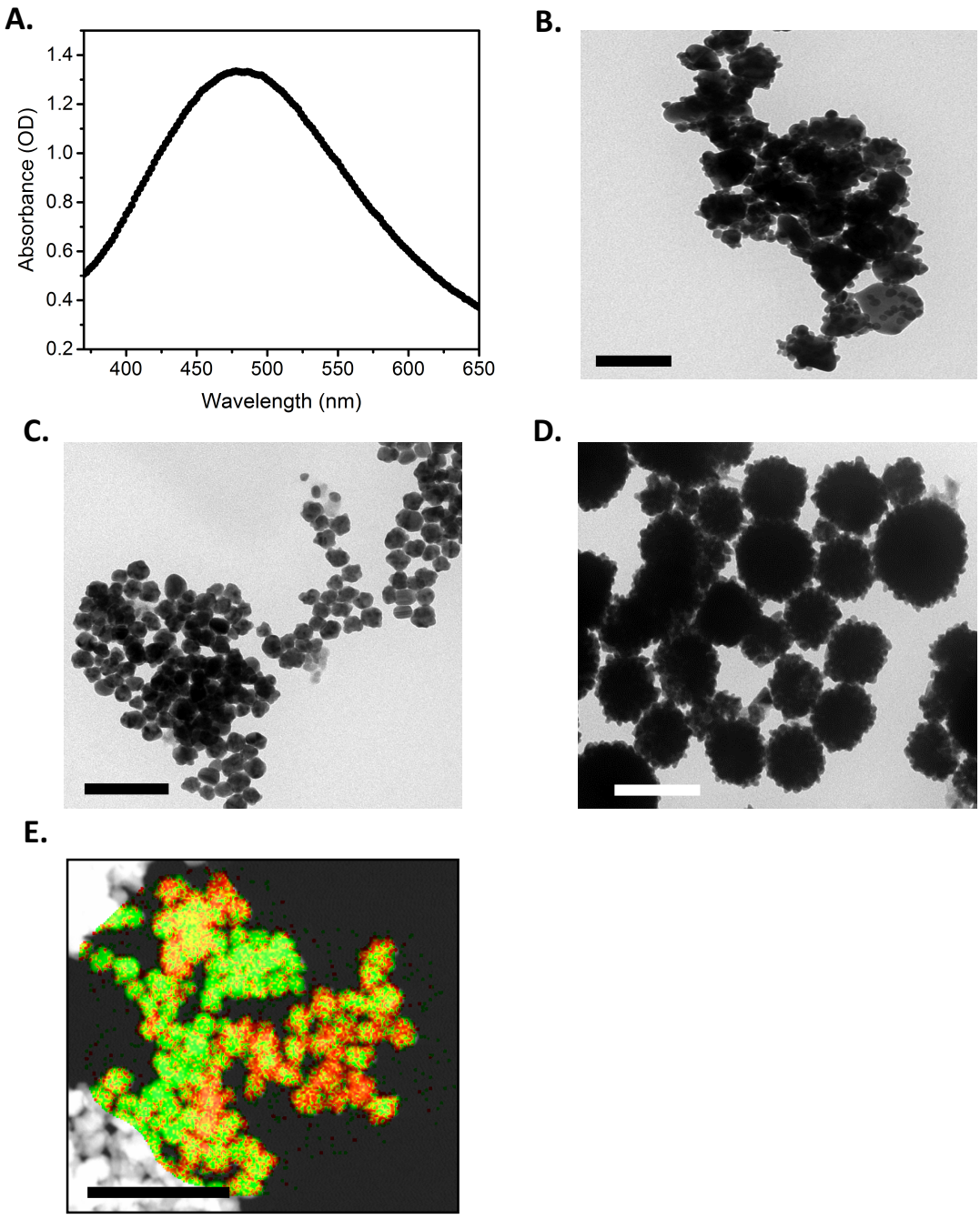

Figure S4 Characterization of bimetallic Au-AgNPs. (A) UV/vis absorption spectra for Au-AgNPs produced using 0.5 mM $HAuCl_4$ as a stressor and 5 mM $AgNO_3$ as a metal target. A characteristic peak at 476 nm indicates presence of bimetallic nanoparticles. (B) TEM image of Au-AgNPs produced using 0.1 mM $HAuCl_4$ as a stressor and 5 mM $AgNO_3$ as a metal target. (C) TEM image of Au-AgNPs produced using 0.5 mM $HAuCl_4$ as a stressor and 5 mM $AgNO_3$ as a metal target. (D) TEM image of Au-AgNPs produced using 1 mM $HAuCl_4$ as a stressor and 5 mM $AgNO_3$ as a metal target. (E) Elemental mapping of Au-AgNPs produced with 0.5 mM $HAuCl_4$ as a stressor and 5 mM $AgNO_3$ as a metal target. Green represents gold, red represents silver, and yellow indicates the presence of both metals. All scale bars are 100 nm.

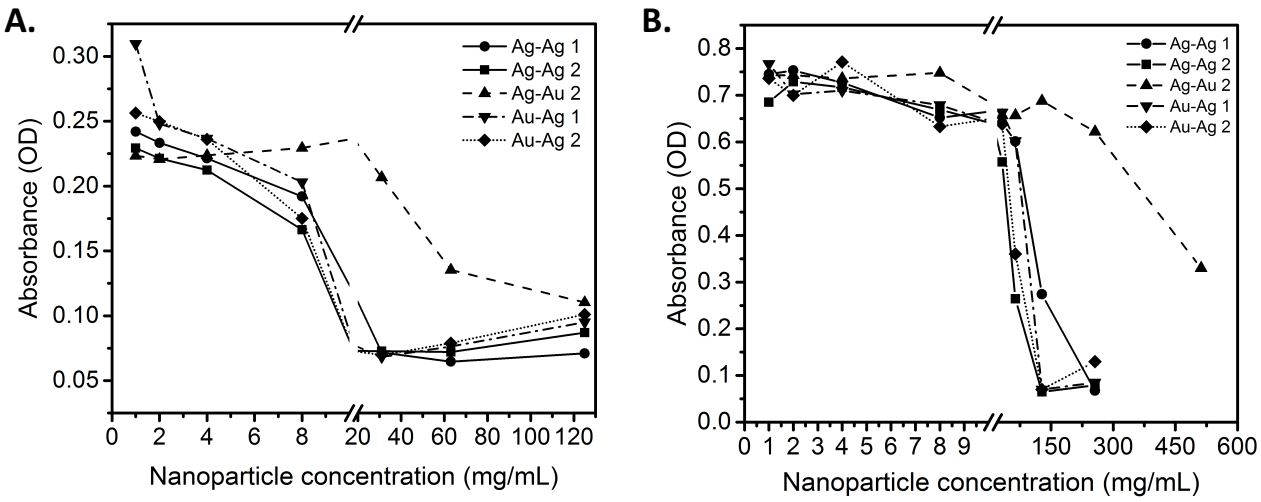

Figure S5 Antimicrobial activity of biosynthetic nanoparticles against *D. radiodurans* and *E. coli*. (A) Microtiter broth dilution results for *D. radiodurans* after 18 hrs of co-incubation with nanoparticles. 1 and 2 denote 0.1 mM metal stressor and 1 mM metal stressor respectively. Ag-Au 2 was unable to inhibit growth even at the highest concentration (125 ug/mL) used in the assay. Ag-Au 1 showed no antimicrobial activity at any of the tested concentrations. (B) Microtiter broth dilution results for *E. coli* after 18 hrs of co-incubation with nanoparticles. Ag-Au 2 was unable to inhibit growth even at the highest concentration (526 ug/mL) used in the assay. Ag-Au 1 showed no antimicrobial activity at any of the tested concentrations.

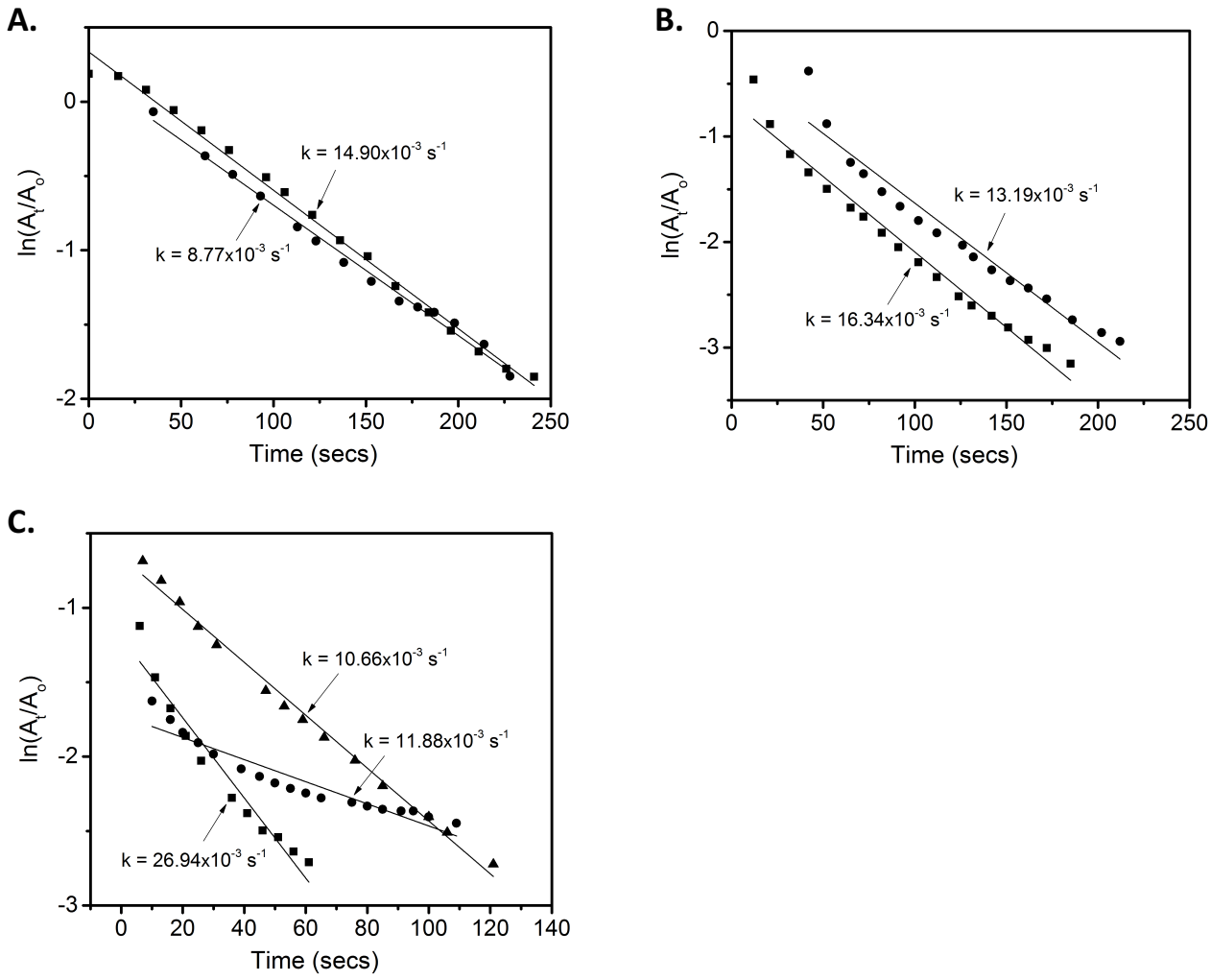

Figure S6 Representative linear fits of 4-nitrophenol reduction to 4-aminophenol. All catalytic assays were performed in triplicate and used to calculate rate constants. (A) Linear fits and rate constants for Ag-AgNPs with Ag-AgNP 1 (circles) and Ag-AgNP 2 (squares) where 1 and 2 denote 0.1 mM metal stressor and 1 mM metal stressor respectively. (B) Linear fits and rate constants for Ag-AuNPs with Ag-AuNP 1 (circles) and Ag-AuNP 2 (squares). (C) Linear fits and rate constants for Au-AgNPs with Au-AgNP 1 (circles), Au-AgNP 2 (squares), and chemically synthesized AuNPs (triangles). The AuNPs were diluted 2-fold compared to the biosynthetic nanoparticles in order to perform the reduction on a measurable time scale.

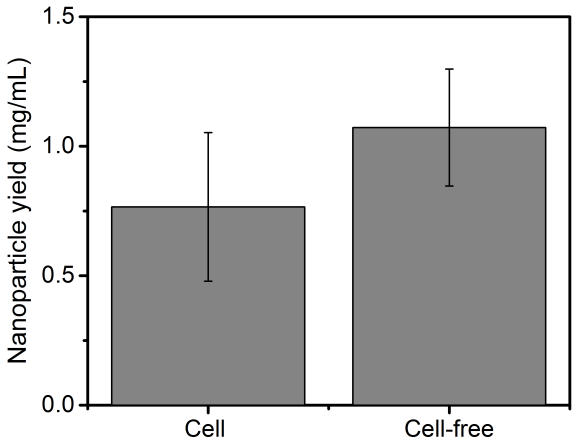

Figure S7 Effect of cell-free reduction on nanoparticle yield is ablated with increasing stress concentration. Silver nanoparticle yield data is shown for cell-free and cell-mediated production after treatment with 1 mM $AgNO_3$ as a stressor and 5 mM $AgNO_3$ as a metal target. There is no statistical difference between the two conditions. Data represents mean ± standard deviation of three replicates.