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Electronic Supplementary Information

Biosynthesis of Silver Nanoparticles from Silver(I) Reduction by the Periplasmic Nitrate Reductase c-type Cytochrome Subunit NapC in a Silver Resistant *E .coli*

Iris Wing-Shan Lin, Chun-Nam Lok* and Chi-Ming Che[*]

Department of Chemistry, State Key Laboratory of Synthetic Chemistry, HKU Shenzhen Institute of Research and Innovation, and Chemical Biology Centre, The University of Hong Kong, Pokfulam, Hong Kong.
*****E-mail: C.M. Che, cmche@hku.hk; C.N. Lok, cnlok@hku.hk

Materials and methods

Bacteria, growth condition and protein expression

Silver-sensitive (116S) and silver-resistant strain 116AR of *E. coli* was kindly provided by Dr. Xian-Zhi Li (1). The deletion mutant 116AR Δ*cusF* and 116AR Δ*cusCFBA* were constructed as previously described.(2) All *E. coli* deletion mutant strains (Keio collection) and NapC expression plasmid (ASKA(-) clone) were obtained from National Bio-resource project, Japan. The *E. coli* strain 116AR was used for all mutation experiments. Standard P1 transduction was used for the construction of c-type cytochrome gene deletion mutant strains (3).

E. coli strains were grown in Luria-broth (LB) medium or M9 minimal medium (12.8 g/L $Na_2HPO_4{\cdot}7H_2O$, 3 g/L KH_2PO_4, 0.5 g/L NaCl, 1 g/L NH_4Cl) supplemented with 0.4 % glucose, 2 mM $MgSO_4$ and 0.1 mM $CaCl_2$. Ampicillin (50 μg/mL), kanamycin (50 μg/mL) or chloramphenicol (25 μg/mL) was added to the medium where necessary for bacterial selection and maintenance.

Expression of NapC in 116AR*Δnap*C strain was induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG**,** 0.05 mM) to the bacteria culture ($O.D._{600}$ ~0.8) at 20 ^oC with shaking speed of 200 rpm. The protein expression level by IPTG induction was examined by immunoblot analysis using anti-His tag antibody. Expression plasmid for *ccm* A-H (pEC86) was kindly provided by Prof. Linda Thöny-Meyer, Laboratory for Biomaterials, Empa, Switzerland.(4)

Biosynthesis of silver nanoparticles

The biosynthesis of silver nanoparticles (nano-Ag) was achieved by inoculating overnight culture of 116AR *E. coli* with 10 volumes of M9 minimal medium in the presence of $AgNO_3$ (300 μM). Anaerobic cultures were set up by filling the medium to the rim of 2 mL microtubes with the caps tightly closed. The bacteria was allowed to grow overnight at 37 ^oC with shaking at 200 rpm. Bacterial suspension with amber coloration was obtained and absorption peak at around 440 nm was observed, indicating the presence of nano-Ag with typical surface plasmon resonance (SPR) absorption. In some experiments, the overnight culture of bacteria grown to stationary phase in M9 medium was replenished with the same volume of fresh M9 medium, incubated with $AgNO_3$ (300 μM) at 37 ^oC with shaking under anaerobic condition, and the biosynthesis of nano-Ag was followed.

Transmission electron microscopy (TEM), Energy dispersive X-ray analysis (EDX) and selected area electron diffraction (SAED)

Sample preparations for TEM experiments were performed as described in literature (5). Bacterial culture was fixed in a solution of glutaraldehyde (2.5 %) in distilled water at room temperature. Cell pellets were collected by centrifugation (1,500 g, 10 min) and washed three times with sucrose washing buffer (0.1 M sodium cacodylate buffer, 0.1 M sucrose, pH 7.4). The pellets were not stained with OsO_4. The cell pellets were gradually dehydrated with 30, 50, 70 and 95 % ethanol for 10 min, respectively and followed by treatment with absolute ethanol for 20 min for three times. The dehydrated pellets were embedded in 1:1 mixture of absolute ethanol/Agar 100 epoxy resin overnight and polymerized in 100 % Agar 100 epoxy resin at 60 ^oC for 2 days. Thin sections were mounted on Cu grids. Micrographs were taken with Philips CM100 TEM. EDX spectra and SAED patterns were recorded with FEI Tecnai G2 20 S-TWIN Scanning Transmission Electron Microscope.

Characterization of periplasmic nano-Ag released by osmotic shock

Isolation of periplasmic fraction by osmotic shock was performed according to standard protocol.(6) A solution of overnight 116AR culture (100 mL) was incubated with 500 μM $AgNO_3$ under anaerobic condition. The bacterial culture was pelleted by centrifugation at 5,000 g for 10 min at 4 ^{o}C. The pellets were re-suspended in 8 mL of the solution containing 20 % w/v sucrose and 0.03 M Tris-HCl pH 8.0 followed by the addition of 2 mL of 5 mM sodium EDTA (pH 8.0). The cell suspension was mixed at 180 rpm for 10 min at room temperature. The re-suspended cells were pelleted by centrifugation at 5,000 g for 10 min at 4 ^{o}C. 10 mL of ice-cold water was added to the cell pellet and mixed at 180 rpm for 10 min at 4 ^{o}C. The nano-Ag solution was collected by centrifugation at 10,000 g for 10 min at 4 ^{o}C and was mounted on Cu grids. Micrographs were taken with Philips CM100 TEM.

References

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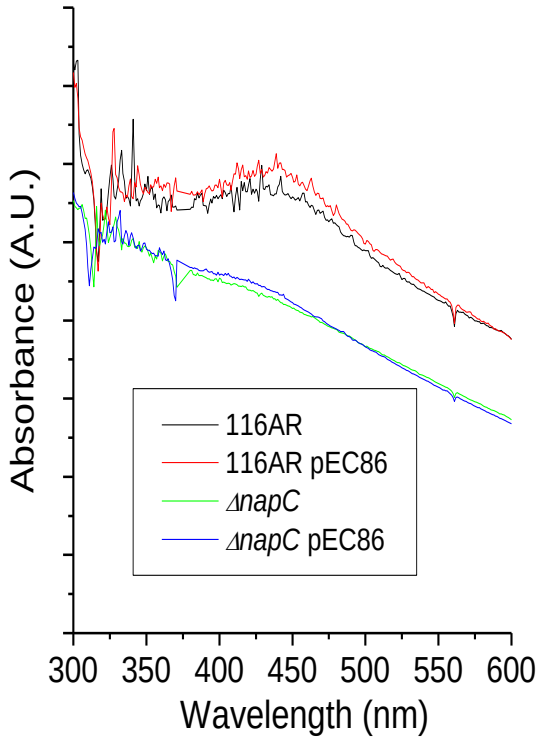

Fig. S1 Effect of expression of cytochrome c maturation proteins (Ccm) on biosynthesis of nano-Ag in 116AR *E. coli*. 116AR and 116AR∆*napC E. coli* were transformed with expression plasmid for *ccm* A-H (pEC86). The overnight culture of bacteria grown to stationary phase in M9 medium was replenished with the same volume of fresh M9 medium, incubated with 300 μM $AgNO_3$ at 37 ^{o}C with shaking under anaerobic condition, and the biosynthesis of nano-Ag was followed by measuring the absorption peak at around 440 nm which indicates the presence of nano-Ag with typical SPR absorption.

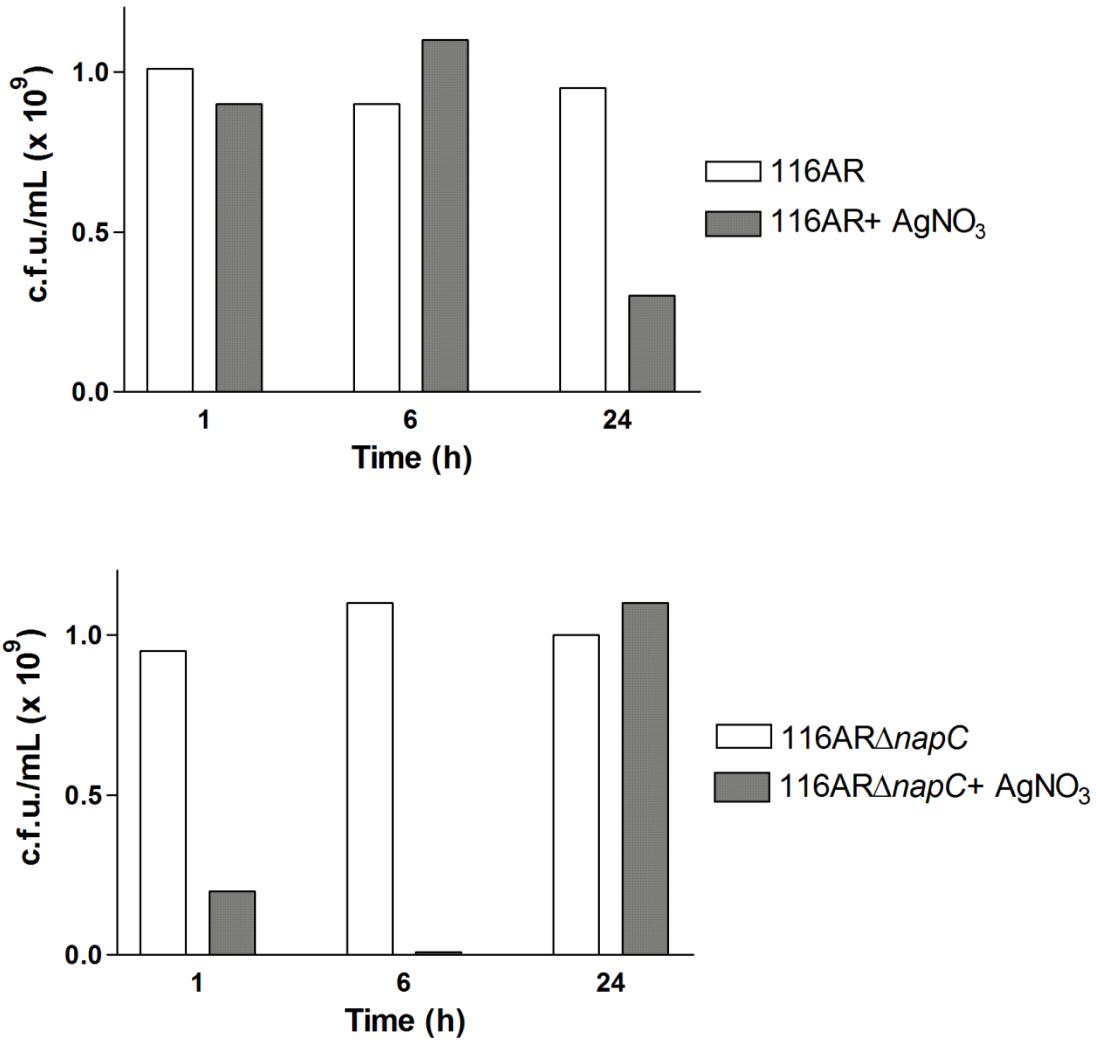

Fig. S2 Effect of *napC* deletion on the colonial growth of 116AR *E. coli* in the presence or absence of $AgNO_3$. 116AR (upper panel) and 116AR*Δnap*C *E. coli* (lower panel) were grown overnight to stationary phase in M9 medium, replenished with the same volume of fresh M9 medium and incubated with 300 μM $AgNO_3$ at 37^{o}C with shaking under anaerobic condition. The colonial growth of the bacteria on agar plate was measured.