

Biocatalytic synthesis of gold nanoparticles with cofactor regeneration in recombinant *Escherichia coli* cells

Supplementary Information

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Experimental details

1. Preparation of glycerol dehydrogenase (GLD)

Recombinant *E. coli* BL21 strains (EMD Biosciences, San Diego, CA, USA) harboring the plasmid vector pET22b(+) encoding GLD¹ were cultivated in 40 mL of LB medium (tryptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L) supplemented with ampicillin (100 mg/L) in a shaking incubator at 37 °C. The overnight culture was used to inoculate 1 L of LB medium supplemented with ampicillin (100 mg/L). The cells were grown at 37 °C to an optical density (OD₆₀₀) of 0.6. When the OD₆₀₀ reached this value, the temperature was lowered to 15 °C, IPTG was added to the medium to a final concentration of 0.5 mM, and the culture was grown for a further 16 h. The cells were harvested by centrifugation at 5800 g for 10 min at 4 °C and washed with a washing buffer (50 mM Tris-HCl (pH 7.4), 100 mM KCl, 1 mM EDTA) three times. To extract the GLD from cells, the washed cells were recollected by centrifugation at 5800 g for 10 min at 4 °C. The pellet was resuspended in 25 mL washing buffer and then sonicated for 15 min on ice with a cooling period of 5 min after the first 5 min and next 5 min. After centrifugation at 5800 g for 10 min and 20800 g for 15 min at 4 °C, the supernatant was filtered through a 0.45 µm pore size membrane filter followed by a 0.25 µm pore size membrane filter (Millipore, Billerica, Massachusetts, USA). The GLD in the supernatant was purified using a Ni-NTA column (HisTrap HP column, GE Healthcare, Uppsala, Sweden) following the manufacture's protocol. The solvent was changed to PBS (pH 7.4) using a PD-10 column (GE Healthcare, Uppsala, Sweden). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out with the sample (Fig. S1).

2. Enzymatic activity analysis of GLD and the effects of the solvent conditions

Activity measurement of prepared GLD was carried out using a UV/Vis spectrophotometer (Ubest-570, JASCO, Tokyo, Japan). The reaction media was composed of GLD (1 µM), NAD⁺ (Oriental Yeast Co., Ltd., Tokyo, Japan) (400 µM) and glycerol (Kishida, Osaka, Japan) (50 mM) in

an aqueous solution (50 mM Gly-NaOH, pH 9.5). The catalytic activity of GLD was monitored by an increase in the absorbance at 340 nm, which arises from the production of NADH by the GLD-mediated enzymatic reduction at 25 °C (Fig. S2).

2. Preparation of the resting *E. coli* cells harboring GLD

Recombinant *E. coli* BL21 cells harboring pET-GLD were cultivated in 40 mL of LB medium (tryptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L) supplemented with ampicillin (100 mg/L) in a shaking incubator at 37 °C and 200 rpm. The overnight culture was transferred to 1 L of LB medium supplemented with ampicillin (100 mg/L). The cells were grown at 37 °C to an optical density (OD₆₀₀) of 0.6. When the OD₆₀₀ reached this value, the temperature was lowered to 15 °C, and the culture medium was divided into two equal parts. IPTG was added to the one part to a final concentration of 0.5 mM; the other part was used without IPTG induction. Both cultures were incubated for a further 16 h. The cells were harvested by centrifugation at 5800 g for 10 min at 4 °C and washed with 50 mM Tris-HCl (pH 7.4) three times, respectively. The washed cells were recollected by centrifugation, frozen in liquid nitrogen, and lyophilized using a freeze-drying apparatus (FD-5N, EYELA, Tokyo, Japan) for 24 h. Resting cells obtained with or without IPTG induction were employed as the resting cells for Au NPs synthesis and were stored at –80 °C prior to use.

3. Characterization of Au NPs

After the synthesis of Au NPs by the *E. coli* sample (Fig. 3-i), the cells were collected by centrifugation at 5800 g for 10 min at 4 °C. The pellets were resuspended in phosphate-buffered saline (PBS, pH 7.4), then subjected to sonication for 15 min on ice with a cooling period of 5 min after the first 5 min and next 5 min. The obtained suspended solution containing Au NPs was directly observed by TEM. For TEM analysis, all samples were placed on carbon coated copper grids and were fixed on TEM grids under vacuum conditions (JEM-2010, JEOL, Tokyo, Japan). TEM experiments were performed at an acceleration voltage of 120 kV. The size of the NPs was manually measured and the sizes of 100 particles were recorded. The particle size distribution of the Au NPs prepared in vitro by GLD-mediated cofactor regeneration was obtained with a Zetasizer Nano-ZS (Malvern, Worcestershire, UK) at 25 °C.

Supplementary Reference

1. H. Ichinose, N. Kamiya, M. Goto, *Biotechnol. Prog.*, 2005, **21**, 1192.

Supplementary Figures

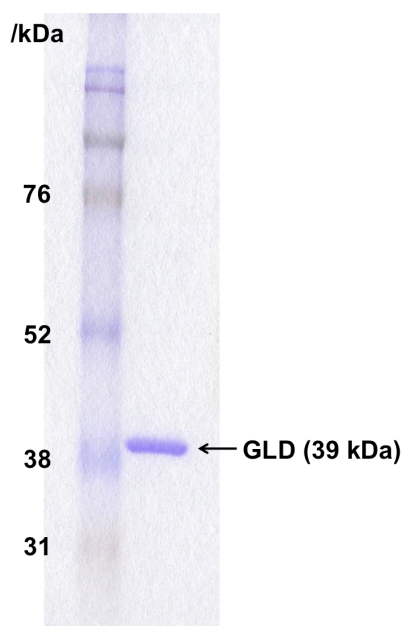


Fig. S1 SDS-PAGE (12.5% polyacrylamide) analysis of the purified GLD sample.

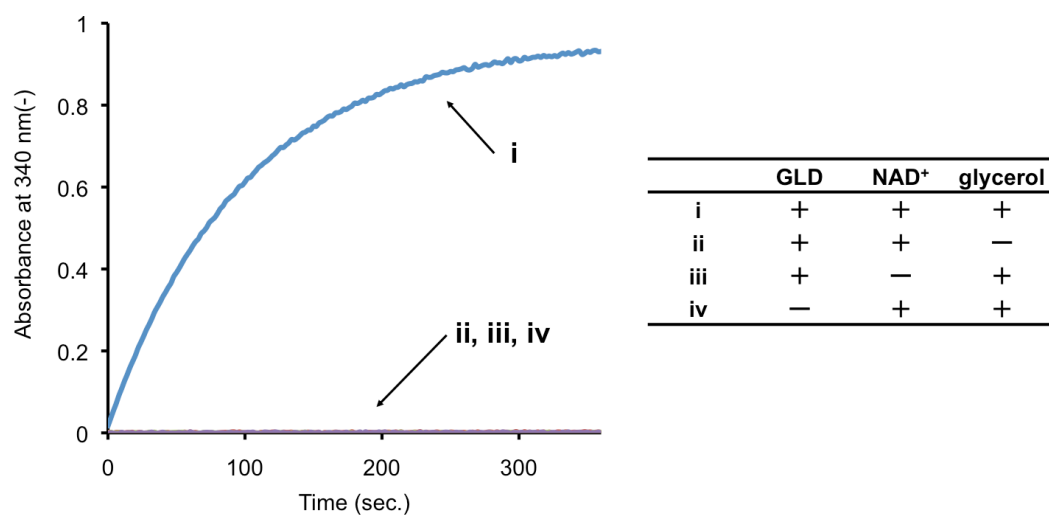


Fig. S2 Spectroscopic analysis of the regeneration of NADH by the GLD enzymatic reaction.

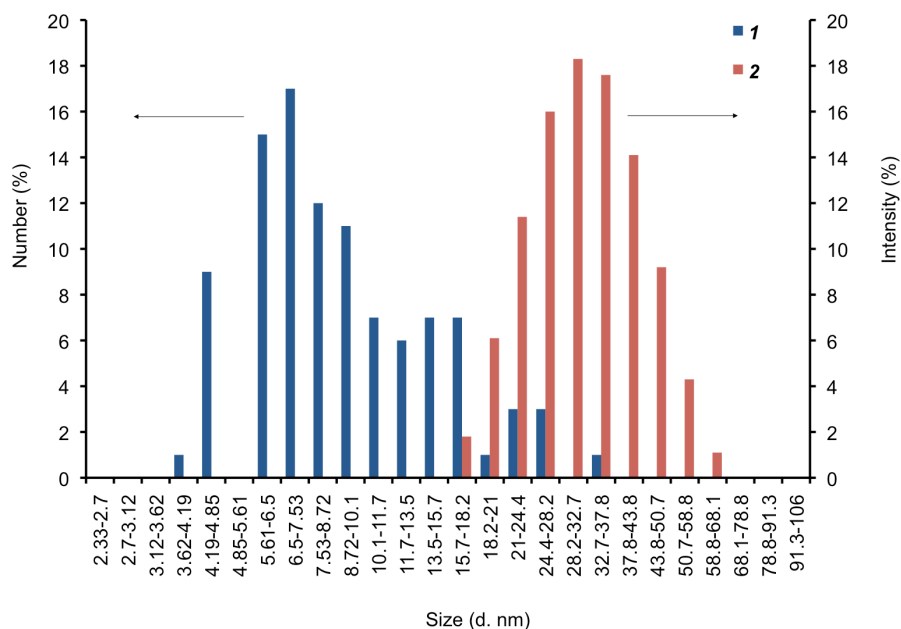


Fig. S3 Particle size distribution of Au NPs corresponding to Fig. 2(b). Size distributions of the Au NPs were created manually from the TEM images (1, $n = 100$) and by DLS analysis (2), in which the scatter diagram (Fig. 2c) was converted into a bar diagram. The width of the x-axis corresponds to a single logarithmic axis of the DLS results.

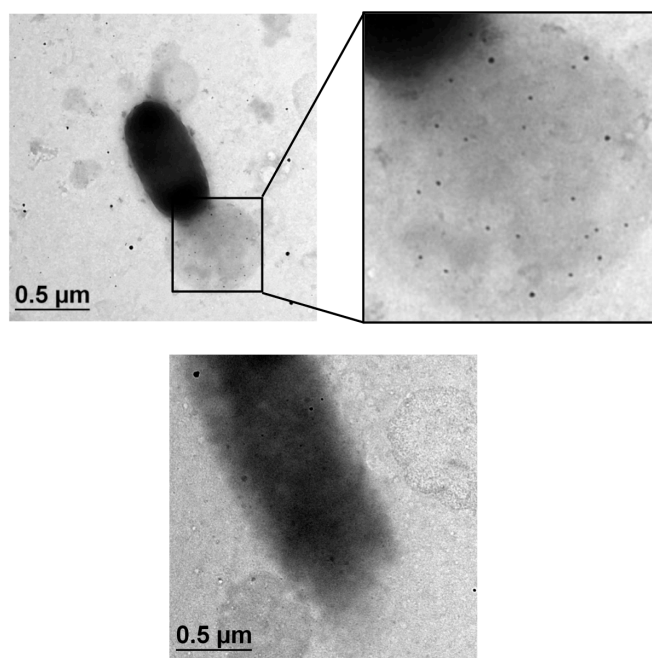


Fig. S4 TEM images of a recombinant *E. coli* cell in which GLD was over-expressed. It is likely that the upper photo shows a snapshot of the leakage of Au NPs from the cytoplasm.

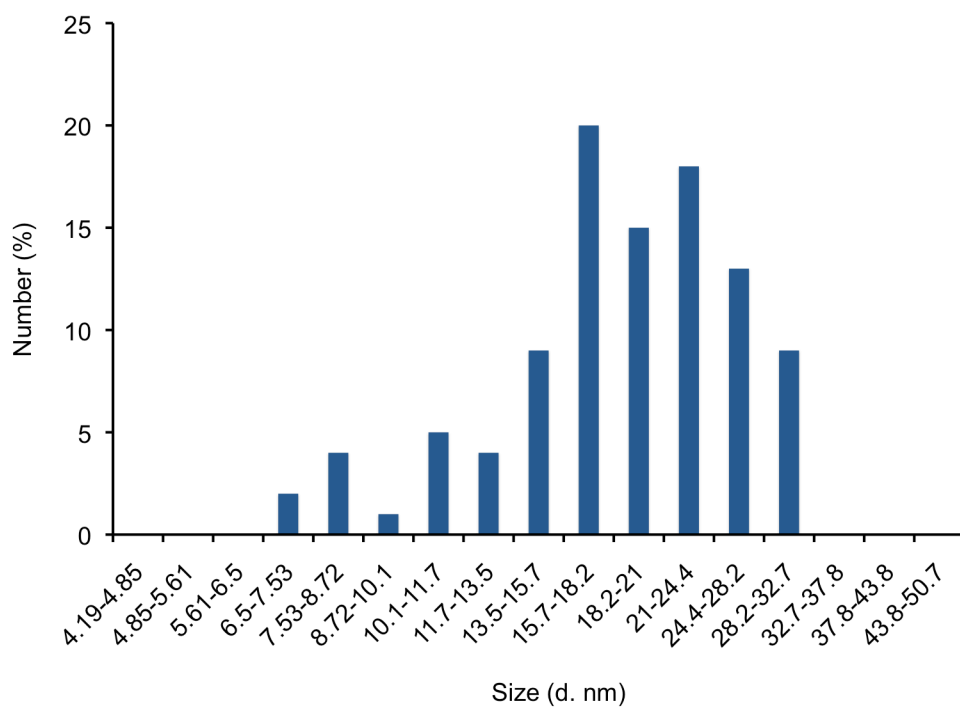


Fig. S5 Particle size distribution of Au NPs corresponding to Fig. 4b and c. The size distribution of the Au NPs was manually obtained using the TEM images. The width of the x-axis is the same as used in Fig. S3.