Enzymatic self-sacrificial display of an active protein on gold nanoparticles

Supplementary Information

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Experimental Details

1. Preparation of glycerol dehydrogenase from *Bacillus stearothermophilus* (BsGLD)

To express the recombinant BsGLD proteins, the expression plasmids were extracted from *E. coli* JM109 and transformed into *E. coli* BL21 (DE3). After the transformation, single colonies were selected from the agar plates (which were supplemented with 100 μg/mL of ampicillin), and were inoculated in 20 mL of Luria-Bertani (LB) medium that contained 100 μg/mL of ampicillin. The media were cultured overnight at 37°C in a shaking incubator. The cultured media were poured into 1 L of LB media containing 100 μg/mL of ampicillin, and then further cultured at 37°C until the optical density at 600 nm (OD_{600}) reached 0.5–0.6. The expression of the BsGLD proteins was subsequently induced *via* the addition of isopropyl *β*-D-thiogalactoside at a final concentration of 0.5 mM, and this was followed by shaking for 16 h at 25°C. The cells were then harvested using 10 min of centrifugation at 5800*g* and 4°C. The cells were washed three times, and then resuspended in a washing buffer (50 mM Tris-HCl, 100 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, pH 7.4). The suspension was frozen using liquid nitrogen, and stored at −80°C. The frozen cell suspensions were completely thawed on ice, and then subjected to 3 × 5 min of sonication, with 5-min intervals between the treatments. The supernatant was collected using 10 min of centrifugation at 5800*g* and 4°C, and this was followed by 15 min of further centrifugation at 20800*g* and 4°C. The supernatant was filtered through 0.45 μm and, subsequently, 0.25 μm pore-size membrane filters. The filtered supernatants were initially applied to an Ni-NTA column that was equilibrated using binding buffer (20 mM Tris-HCl, 500 mM NaCl, 35 mM imidazole, pH 7.4), and the column was washed using five column bed volumes of the binding buffer. The recombinant proteins were then eluted using elution buffer (20 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole, pH 7.4). The eluates were monitored by measuring the absorbance at 280 nm, and protein fractions were collected. The solvent was then changed to 20 mM Tris-HCl (pH 7.4), using a PD-10 column as a desalting and buffer exchange column. The protein concentrations of the obtained protein solutions were measured using a BCA assay, with bovine serum albumin as a standard. The purities of the recombinant proteins were determined using SDS-PAGE analysis.

The amino acid sequences of the BsGLDs were as follows:

1: BsGLD-His_6

MGSENLYFQGGGGSGGGSMAAERVFISPAKYVQGKNVITKIANYLEGIGNKTVVIADEIVWKIAGHTIVNELKKGN
IAAEEVVFSGEASRNEVERIANIARKAEAAIVIGVGGGKTLDTAKAVADELDAYIVIVPTAASTDAPTSALSVIYS
DDGVFESYRFYKKNPDLVLVDTKIIANAPPRLLASGIADALATWVEARSVIKSGGKTMAGGIPTIAAEAIAEKCEQ
TLFKYGKLAYESVKAKVVTPALEAVVEANTLLSGLGFESGGLAAAHAIHNGFTALEGEIHHLTHGEKVAFGTLVQL
ALEEHSQQEIERYIELYLSLDLPVTLEDIKLKDASREDILKVAKAATAEGETIHNAFNVTADDVADAIFAADQYAK
AYKEKHRKHHHHHH

2: BsGLD-His_6Cys

MGSENLYFQGGGGSGGGSMAAERVFISPAKYVQGKNVITKIANYLEGIGNKTVVIADEIVWKIAGHTIVNELKKGN
IAAEEVVFSGEASRNEVERIANIARKAEAAIVIGVGGGKTLDTAKAVADELDAYIVIVPTAASTDAPTSALSVIYS
DDGVFESYRFYKKNPDLVLVDTKIIANAPPRLLASGIADALATWVEARSVIKSGGKTMAGGIPTIAAEAIAEKCEQ
TLFKYGKLAYESVKAKVVTPALEAVVEANTLLSGLGFESGGLAAAHAIHNGFTALEGEIHHLTHGEKVAFGTLVQL
ALEEHSQQEIERYIELYLSLDLPVTLEDIKLKDASREDILKVAKAATAEGETIHNAFNVTADDVADAIFAADQYAK
AYKEKHRKHHHHHHC

3: pG-BsGLD-His_6Cys

MWSHPQFEKGSTYKLVINGKTLKGETTTEAVDAATAEKVFKQYANDNGVDGEWTYDDATKTFTVTEGSENLYFQGG
GGSGGGSMAAERVFISPAKYVQGKNVITKIANYLEGIGNKTVVIADEIVWKIAGHTIVNELKKGNIAAEEVVFSGE
ASRNEVERIANIARKAEAAIVIGVGGGKTLDTAKAVADELDAYIVIVPTAASTDAPTSALSVIYSDDGVFESYRFY
KKNPDLVLVDTKIIANAPPRLLASGIADALATWVEARSVIKSGGKTMAGGIPTIAAEAIAEKCEQTLFKYGKLAYE
SVKAKVVTPALEAVVEANTLLSGLGFESGGLAAAHAIHNGFTALEGEIHHLTHGEKVAFGTLVQLALEEHSQQEIE
RYIELYLSLDLPVTLEDIKLKDASREDILKVAKAATAEGETIHNAFNVTADDVADAIFAADQYAKAYKEKHRKHHH
HHHC

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

The catalytic activity of the prepared BsGLD was measured using a UV/Vis spectrometer (Ubest-570, JASCO, Tokyo, Japan), after BsGLD (1 μM), NAD^+ (400 μM), and glycerol (100 mM) were added to an aqueous solution (50 mM sodium phosphate buffer, pH 8.0) at 25°C. The catalytic activity of BsGLD was assessed by the NADH formation that is followed by the absorbance at 340 nm (Figure S2). In addition, the BsGLD activity was measured under various buffer and pH conditions after BsGLD (1 μM), NAD^+ (400 μM), and glycerol (100 mM) were added to an aqueous solution (50 mM various buffer, pH 6.0–11.0) at 25°C (Figure S3).

3. Preparation of protein-decorated Au NPs

The BsGLD-Au NP conjugates (**1**-, **2**-, and **3**-Au NPs) were synthesized using their enzymatic reaction in the presence of functional proteins. The BsGLDs were added (at a final concentration of 1 μM) to an aqueous solution of NAD^+ (400 μM) and glycerol (100 mM) in 50 mM of Gly-NaOH buffer (pH 9.5). After the addition of $AuCl_4^-$ (0.5 mM), the reaction mixture was incubated at 37°C for 48 h. To purify the resulting Au NPs, the red-colored Au NP solution was centrifuged at 4000*g* for 3 min at 4°C, using a centrifugal filter unit (100 kDa MWCO). The residue was then washed five times using a 20 mM aqueous solution of Tris-HCl (pH 7.4) by repeating the ultrafiltration procedure, and was finally resuspended in the same buffer solution.

4. Characterization of protein-decorated Au NPs

For the transmission electron microscopy (TEM) analysis, all samples were placed on carbon-coated copper grids, and then dried under vacuum conditions. The TEM experiments were performed using a JEM–2010 instrument (JEOL, Tokyo, Japan) operating at 120 kV. The average diameter of the Au NPs was calculated using ImageJ, by measuring the diameters of 169–205 NPs per sample. The particle size was also evaluated using a Zetasizer Nano-ZS (Malvern, Worcestershire, UK) with temperature control. Each run was recorded at 25°C. The bound proteins were analyzed using SDS-PAGE. To remove unbound proteins and concentrate the solutions, the protein-Au NP solutions were applied to a centrifugal filter unit (100 kDa MWCO) at 4°C. Each sample was then resuspended in SDS 2×sample buffer (0.25 M Tris-HCl (pH 6.8), 4 wt% SDS, 20 wt% glycerol, 0.01 wt% bromophenol blue, and 12 wt% 2-mercaptoethanol) and heated at 94°C for 30 min, to detach and denature the bound proteins. The supernatant was loaded on a 12.5% SDS polyacrylamide gel.

5. Evaluation of the target protein binding efficiency of the protein-AuNPs in an enzyme-linked immunosorbent assay (ELISA)

The BsGLD-Au NP conjugates (**1**-, **2**-, and **3**-Au NPs) were prepared using their enzymatic reaction. After incubation for 48 h at 37°C, the functional BsGLD-Au NP solutions were purified via ultrafiltration using a centrifugal filter unit (100 kDa MWCO), and the residue was washed using 20 mM Tris-HCl (pH 7.4). The concentration of Au NPs was measured using UV/vis spectroscopy. The BsGLD-Au NP conjugates were then diluted using 1% casein Na in 1×PBS (pH 7.4, acting as a blocking solution) to give an absorbance of $\lambda_{max,\ SPR\ band} = 0.05$, and the solutions were stored at 4°C.

Ninety-six-well polystyrene plates were coated with 100 μL/well of an aqueous OVA antigen solution (5.0 mg/mL), and then incubated overnight at 4°C. The plates were washed five times for each well, using PBST (0.1 % Tween-20 in PBS), and then blocked with 200 μL/well of casein Na in PBS (1.0 wt%) for 1.5 h at 37°C. Next, the plates were washed again with PBST, and 100 μL/well of a rabbit anti-OVA IgG (0.1 μg/mL) was added; this was followed by incubation for 1.5 h at 37°C. After the plates were washed, 50 μL/well of the

surface-blocked functional BsGLD-Au NPs were added to each well, and the plates were incubated for 1.5 h at 37°C. After the plates were washed, 100 μL/well of an HRP-labeled rabbit anti-mouse IgG antibody (0.1 μg/mL) (Rockland) was added, and the plates were incubated for 1.5 h at 37°C. After washing, 100 μL/well of a tetramethyl benzidine ELISA solution (Sigma or Wako Pure Chemical Industries) was added to each well and incubated for 30 min at 37°C. A total of 50 μL/well of 1.0 M HCl was added, and the end point optical density was measured at 415 nm using a microplate reader (Bio-Tek, Winooski, VT, USA).

Supplementary Data

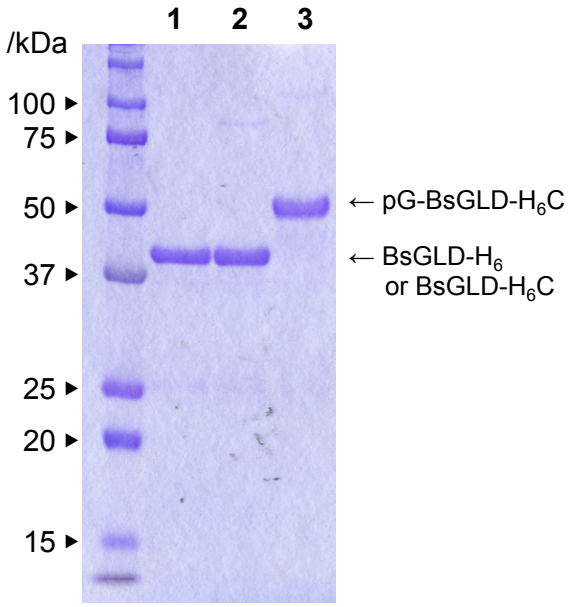

Figure S1 SDS-PAGE (12.5% polyacrylamide) analysis of the purified BsGLDs (**1**–**3**). The theoretical molecular weights of **1**, **2**, and **3** were 42.0, 42.1, and 49.3 kDa, respectively.

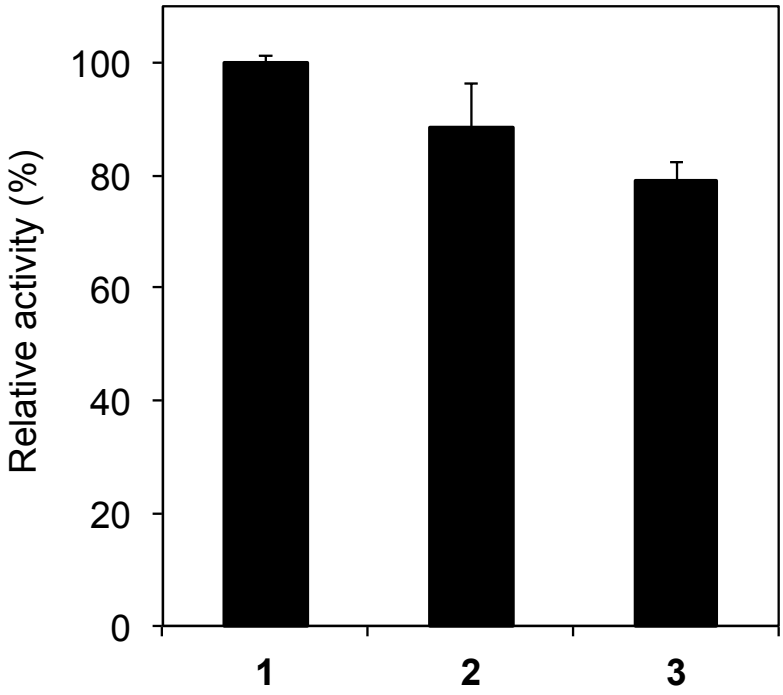

Figure S2 Catalytic activity of the BsGLDs (**1**–**3**).

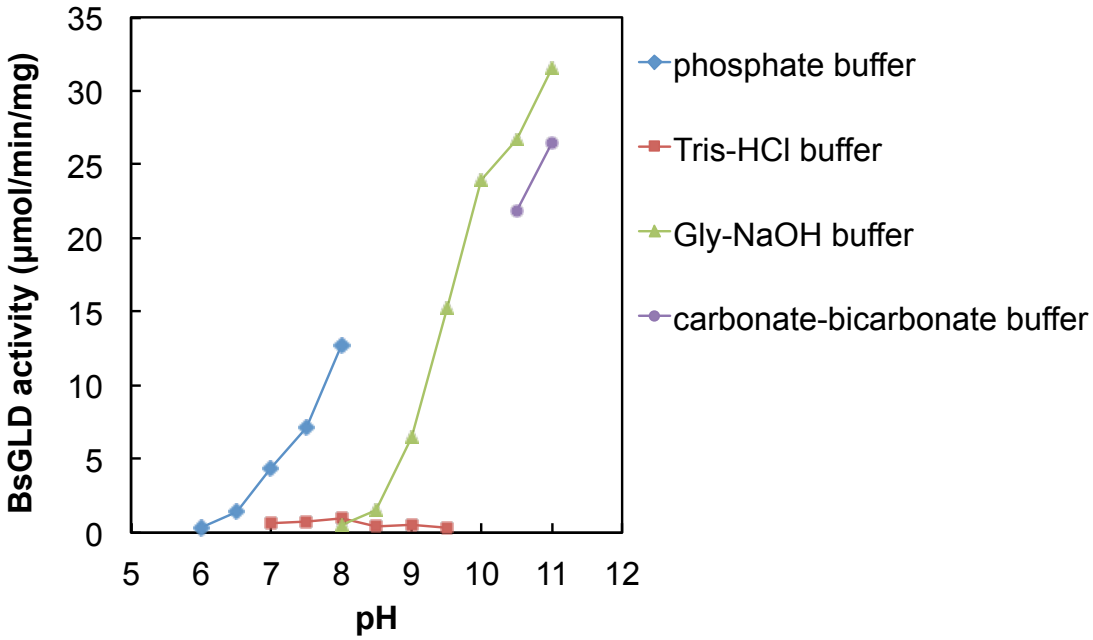

Figure S3 Enzymatic activity of BsGLD **1** under various buffer conditions.

Buffer solution	Entry	pH
Sodium phosphate buffer	a, b, c	7.0, 7.5, 8.0
Tris-HCl buffer	d, e	8.0, 9.0
Glycine-NaOH buffer	f, g, h	8.0, 9.0, 10.0
Carbonate-bicarbonate buffer	i, j	10.0, 11.0

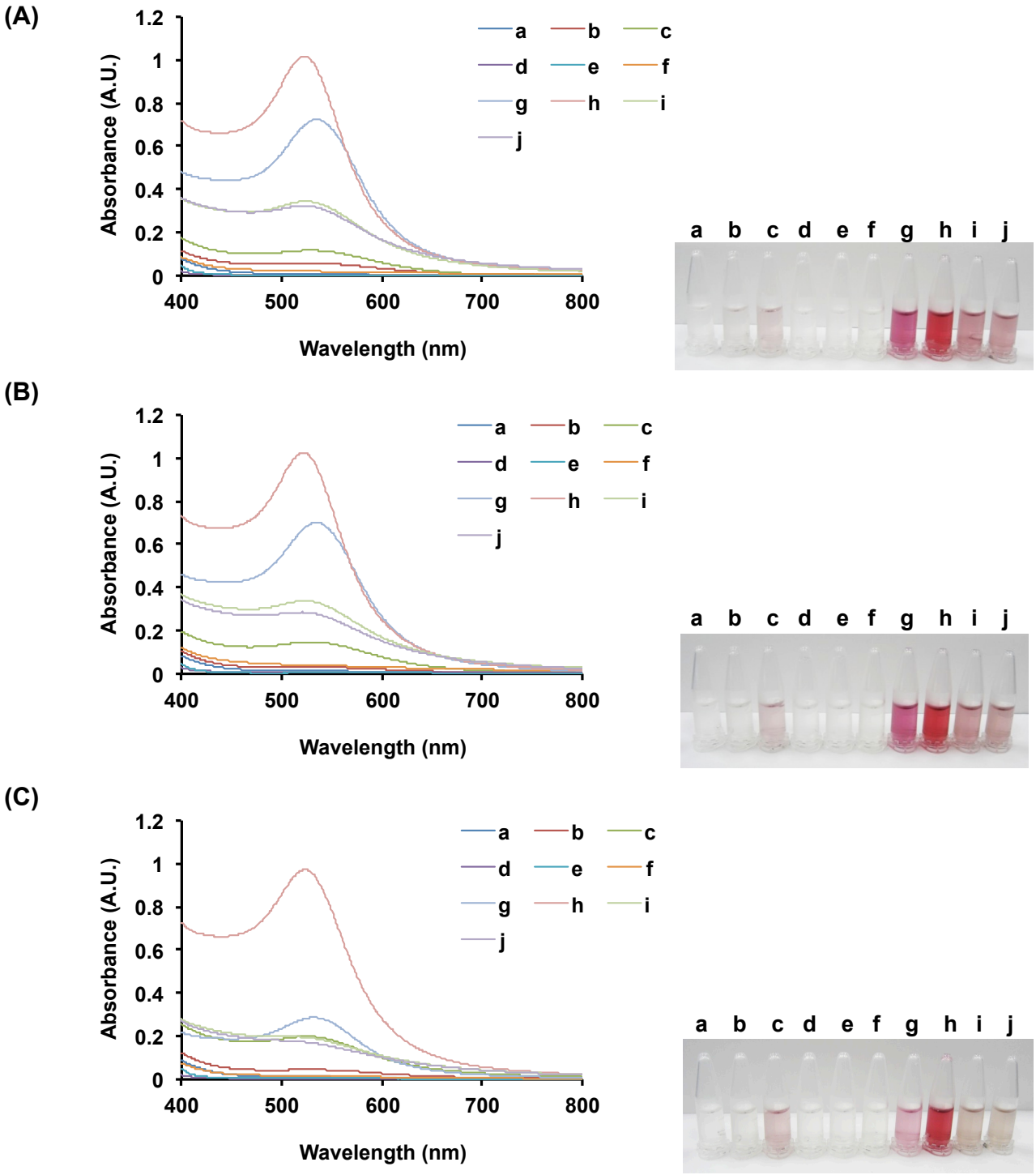

Figure S4 UV-vis spectra and sample images of Au NP solutions synthesized using various buffer solutions and pH values (spectra and images measured and taken after 48 h): with (A) BsGLD **1**, (B) BsGLD **2**, (C) BsGLD **3**. [BsGLD] = 1 μM, [glycerol] = 50 mM, [NAD^+] = 400 μM, [$HAuCl_4$] = 0.25 mM.

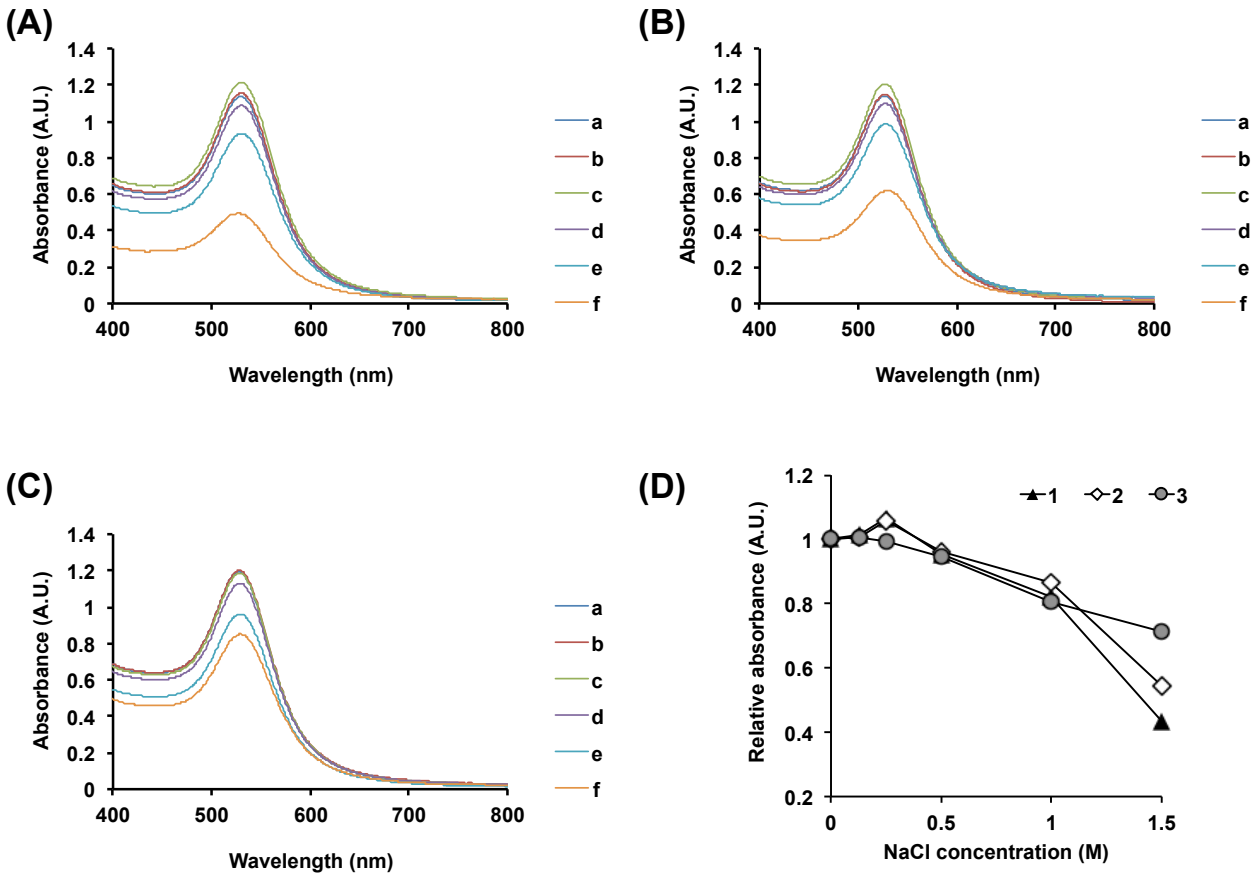

Figure S5 Stability of BsGLD-Au NP conjugates, assessed using UV-vis absorption spectra measured in 50 mM Gly-NaOH buffer (pH 9.5): with (A) BsGLD **1**, (B) BsGLD **2**, (C) BsGLD **3**. The Au NPs were synthesized via an enzymatic BsGLD reaction, which was performed for 48 h. The NaCl concentration in the Au NP solutions was adjusted to (a) 0 mM, (b) 125 mM, (c) 250 mM, (d) 500 mM, (e) 1 M, and (f) 1.5 M, and the absorbance spectra were measured after 2 h. (D) The relative absorbance of these λ_{max} values for the SPR bands versus NaCl concentration.

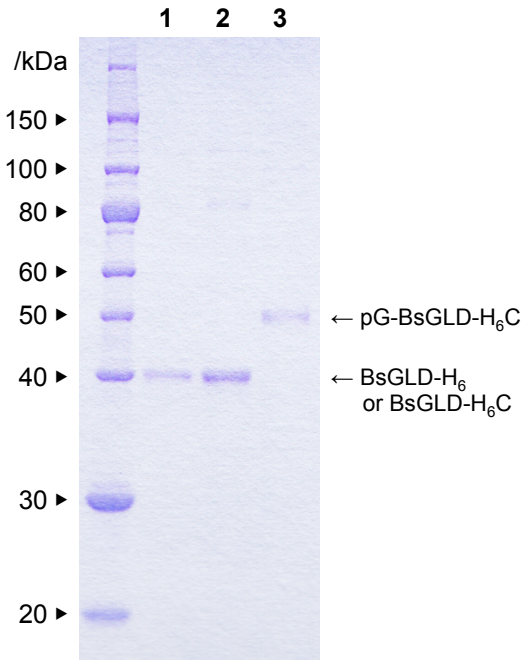

Figure S6 SDS-PAGE analysis of proteins detached from purified **1**-, **2**-, or **3**-Au NPs. The staining bands demonstrated that the proteins were immobilized on the Au NPs.

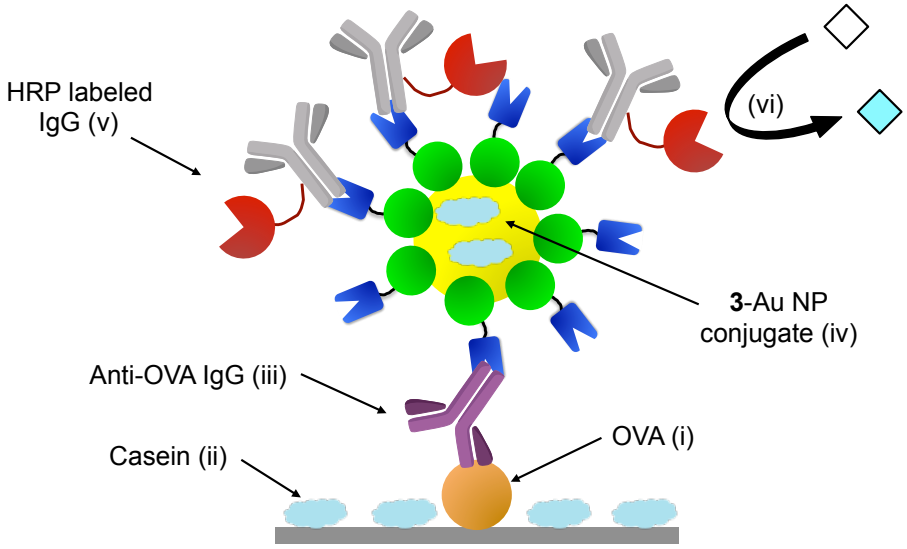

Figure S7 Schematic illustration of the ELISA assay performed in this study.