Electronic supplementary information

Facile, rapid and efficient biofabrication of gold nanoparticles decorated with functional proteins

Kojiro Shimojo,*[a] Teppei Niide,[b] Tomitsugu Taguchi,[c] Hirochika Naganawa,[a] Noriho Kamiya[b,d] and Masahiro Goto[b,d]

[a] *Division of Environment and Radiation Sciences, Nuclear Science and Engineering Directorate, Japan Atomic Energy Agency, Tokai-mura, Ibaraki 319-1195, Japan.*

[b] *Department of Applied Chemistry, Graduate School of Engineering, Kyushu University, 744 Motooka, Fukuoka 819-0395, Japan*

[c] *Quantum Beam Science Directorate, Japan Atomic Energy Agency, Tokai-mura, Ibaraki 319-1195, Japan*

[d] *Center for Future Chemistry, Kyushu University, 744 Motooka, Fukuoka 819-0395, Japan*

*To whom corresponding should be addressed. E-mail: shimojo.kojiro@jaea.go.jp

Experimental details

1. Preparation of peptide-fused ZZ domain (ZZ-Trx-A3 and ZZ-A3)

A gene fragment encoding a fusion protein consisting of the ZZ domain, thioredoxin and A3 peptide tag (ZZ-Trx-A3) was amplified by PCR employing a synthetic vector (pUC57 ZZ-Trx-A3) purchased from BEX Co., Ltd. (Tokyo, Japan). The gene encoding ZZ-Trx-A3 was inserted into the *Nde*I/*Xho*I restriction sites of the pET28 plasmid (pET28-ZZ-Trx-A3). To obtain a fusion protein consisting of only the ZZ domain and the A3 peptide tag (ZZ-A3), the gene encoding the thioredoxin segment of the pET28-ZZ-Trx-A3 construct was digested with *Bam*HI, and the recombinant vector encoding ZZ-A3 (pET28-ZZ-A3) was constructed by self-ligation of the *Bam*HI-digested pET28-ZZ-Trx-A3. The integrity of the constructs was verified by DNA sequencing.

The pET28-ZZ-Trx-A3 or pET28-ZZ-A3 constructs were transformed into the *Escherichia coli* BL21(DE3) (Novagen) strain. The transformants harboring the recombinant plasmid were grown in 40 mL of LB medium (tryptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L) supplemented with kanamycin (50 mg/L) at 37 °C and 200 rpm. The overnight cultures were used to inoculate 1 L of LB medium supplemented with kanamycin (50 mg/L), and the cells were grown at 37 °C and 200 rpm to an optical density (OD_{600}) of 0.6. When the OD_{600} reached a value of 0.6, the temperature was lowered to 25 °C and isopropyl-β-D-thiogalactopyranoside was added to the medium to a final concentration of 0.5 mM. After the cultures were incubated overnight at 25 °C, the cells were harvested by centrifugation at 8000 rpm for 10 min at 4 °C and washed with a washing buffer (25 mM Tris-HCl (pH 7.4), 137 mM NaCl, 2.68 mM KCl, 1 mM EDTA) three times. The washed cells were recollected by centrifugation at 8000 rpm for 10 min at 4 °C. The pellets were resuspended in 25 mL of a buffer (20 mM Tris-HCl (pH 7.4), 500 mM NaCl, 40 mM imidazole, 0.1 mM EDTA) and disrupted by 15 cycles of homogenization (sonication for 1 min and subsequent cooling for 30 sec) on ice. The lysates were centrifuged at 15000 rpm for 10 min at 4 °C. After the supernatants were heated for 10 min at 80 °C, the resulting proteins that had precipitated were removed by centrifugation 15000 rpm for 10 min at 4 °C. The supernatants were filtered through a 0.45 μm pore size membrane filter, followed by a 0.22 μm pore size membrane filter. The target proteins in the supernatants were purified using a

Ni-NTA column (HisTrap HP column, GE Healthcare UK Ltd.) and following the manufacturer's protocol. The eluted fractions containing the target protein were concentrated using Amicon Ultra centrifugal filter units with a 10 kDa cutoff membrane (Millipore), and the aqueous solution was exchanged to TBS (20 mM Tris-HCl (pH 8.0), 100 mM KCl) using a desalting column (PD-10 column, GE Healthcare UK Ltd.). For storage, 50% glycerol solutions of the purified proteins were prepared and stored at –20 °C. The integrity of the target proteins was verified by SDS-PAGE (Figure S1). The concentrations of the proteins were determined using the BCA Protein Assay Kit (Sigma).

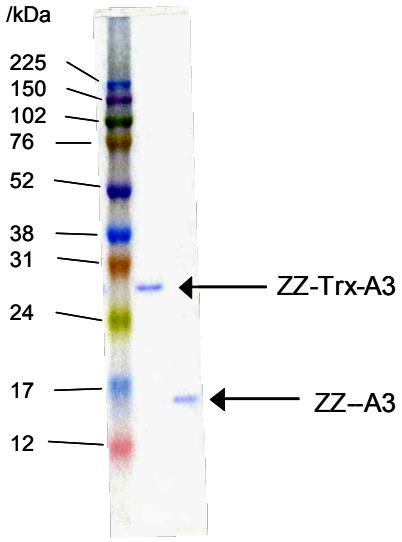


Fig. S1 SDS-PAGE (12.5% polyacrylamide) analysis of the purified ZZ-Trx-A3 and ZZ-A3 fusion proteins.

2. Gold nanoparticles synthesis using a peptide-functionalized protein (ZZ-Trx-A3)

HEPES stock solutions (100 mM) were prepared over the pH range of 3.0 to 8.0, and diluted with ultrapure water to the desired concentration. Next, 5 μL of the ZZ-Trx-A3 stock solution (298 μM) was added to the HEPES buffer (990 μL), followed by the addition of 5 μL of the $HAuCl_4$ solution (50 mM, pH 1.36). The prepared solution was gently shaken for a few seconds and then left undisturbed at room temperature for 3 h. The resulting gold nanoparticle (AuNP) solutions were stored at 4 °C prior to use. The final concentration of the ZZ-Trx-A3 protein was 1.5 μM, and that of $HAuCl_4$ was 0.25 mM. The detailed experimental conditions are summarized in Table S1.

Table S1 Experimental conditions for gold nanoparticle synthesis with proteins

ultrapure water	990–X μL
100 mM HEPES (pH 3.0, 4.0, 5.0, 6.0, 6.5, 7.0 and 8.0)	X = 100 μL (final: 10 mM)
	X = 200 μL (final: 20 mM)
	X = 300 μL (final: 30 mM)
	X = 500 μL (final: 50 mM)
	X = 990 μL (final: 100 mM)
ZZ-Trx-A3	5 μL (final: 1.5 μM)
50 mM $AuCl_4$ in H_2O (pH 1.36)	5 μL (final: 0.25 mM)
Total	1000 μL

3. Characterization of the AuNPs

The ZZ-Trx-A3-coated AuNPs (ZZ–AuNPs) were fabricated according to the procedure described above. UV/Vis adsorption spectra of the ZZ–AuNPs (400–800 nm) were measured using a UV/Vis spectrophotometer (Jasco V-550) or a spectral scanning multimode reader Varioskan Flash 2.4 (Thermo Fisher Scientific). TEM samples were prepared by pipetting a few drops of solution onto plasma-cleaned carbon-coated copper grids. TEM analysis was conducted using a JEOL JEM-2000F (JEOL) at an accelerating voltage of 200 kV. The size of the ZZ–AuNPs was measured from the TEM images. The particle size distribution of the ZZ–AuNPs and proteins (ZZ-Trx-A3, rabbit anti-lysozyme IgG and lysozyme) was obtained with a High Performance Particle Sizer (Malvern) at 25 °C.

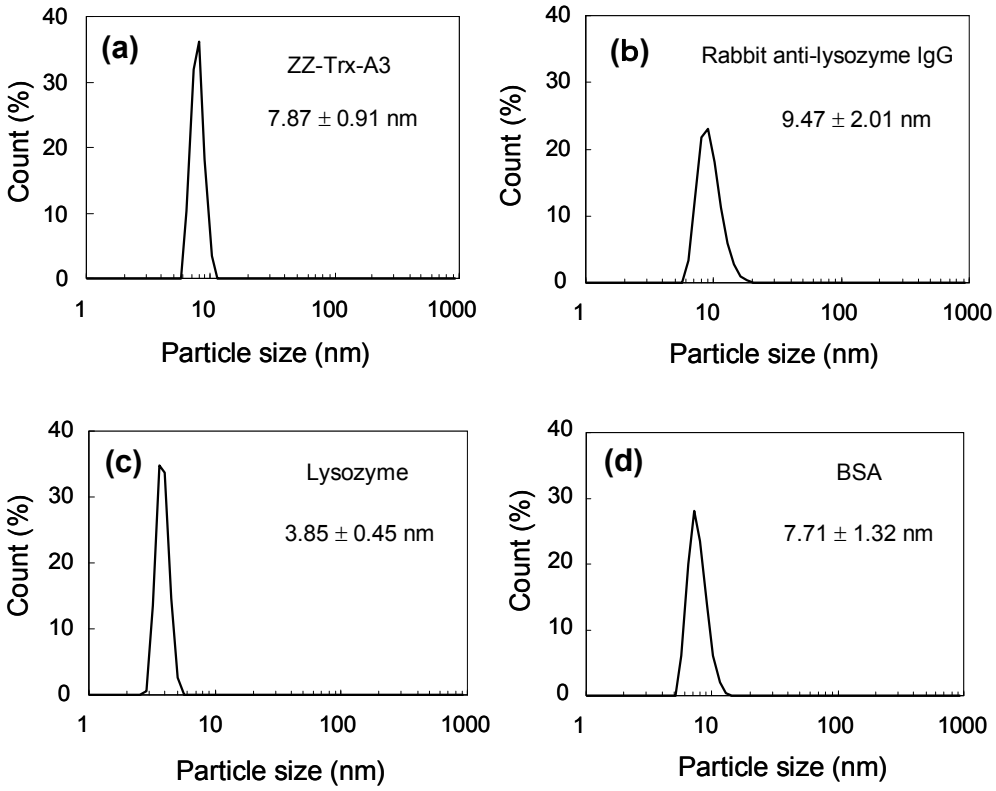


Fig. S2 DLS data of ZZ-Trx-A3 (a), rabbit anti-lysozyme IgG (b), lysozyme (c) and BSA (d).

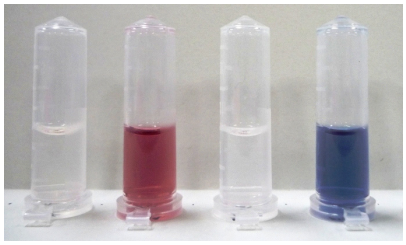


Fig. S3 Effect of buffers on AuNPs biofabrication using the ZZ-Trx-A3 protein. [ZZ-Trx-A3] = 1.5 μM; [buffer] = 10 mM; [$HAuCl_4$] = 0.25 mM.

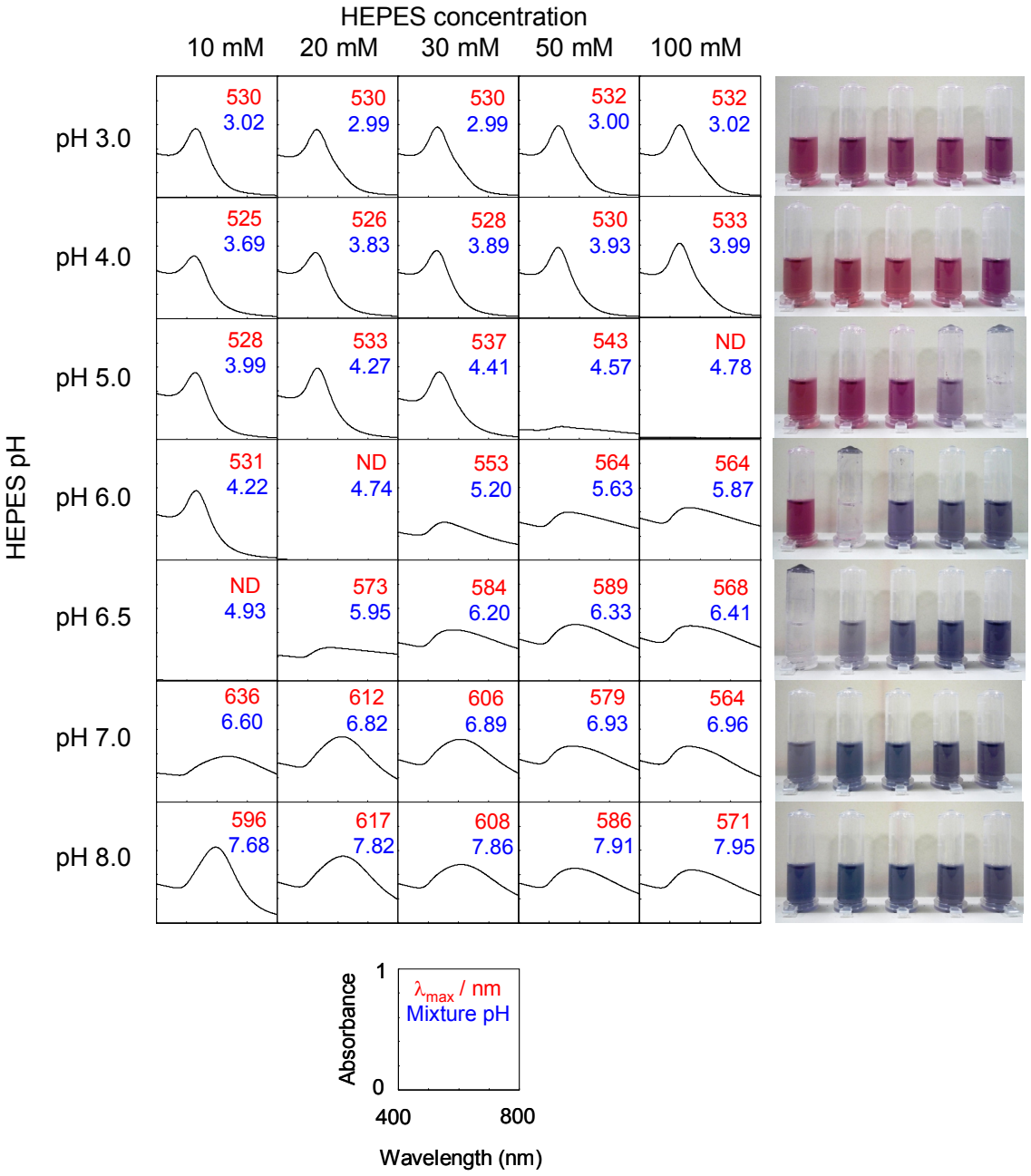
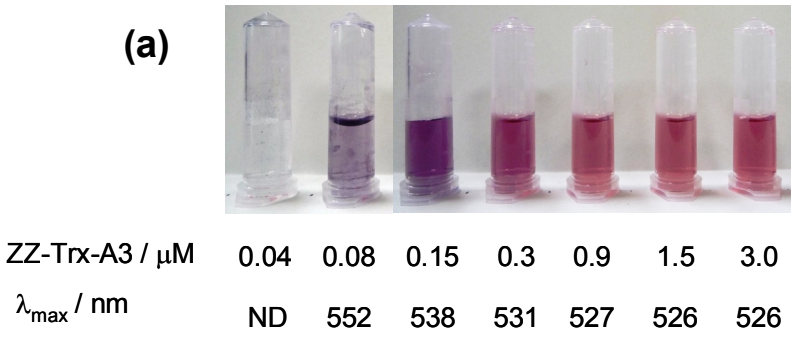


Fig. S4 UV/Vis spectra and macroscopic appearances of AuNP solutions synthesized by varying the HEPES concentration and pH conditions. Mixture pH denotes the final pH value following the addition of a $HAuCl_4$ solution (pH 1.3) to the HEPES buffer. [ZZ-Trx-A3] = 1.5 μM; [$HAuCl_4$] = 0.25 mM. ND: not detectable.



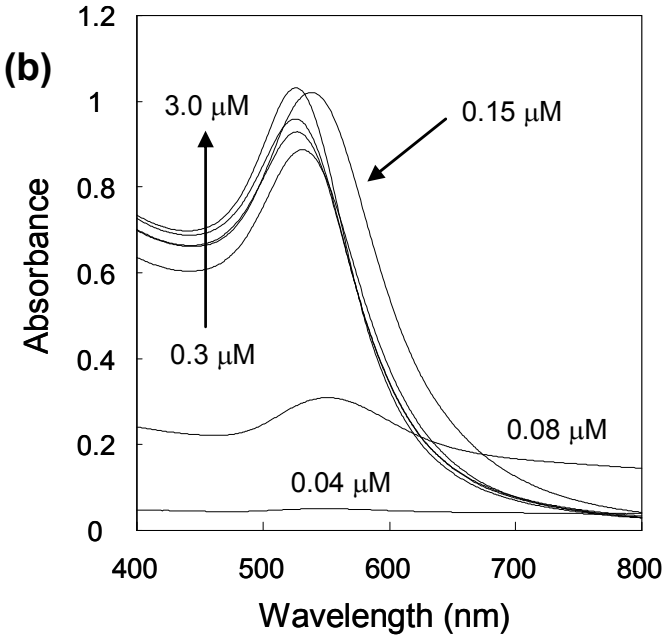


Fig. S5 Influence of the ZZ-Trx-A3 concentration on AuNPs biofabrication. (a) Macroscopic appearance of the AuNP solutions, and (b) corresponding UV/Vis spectra. [HEPES] = 10 mM, pH 4.0; [$HAuCl_4$] = 0.25 mM. ND: not detectable.

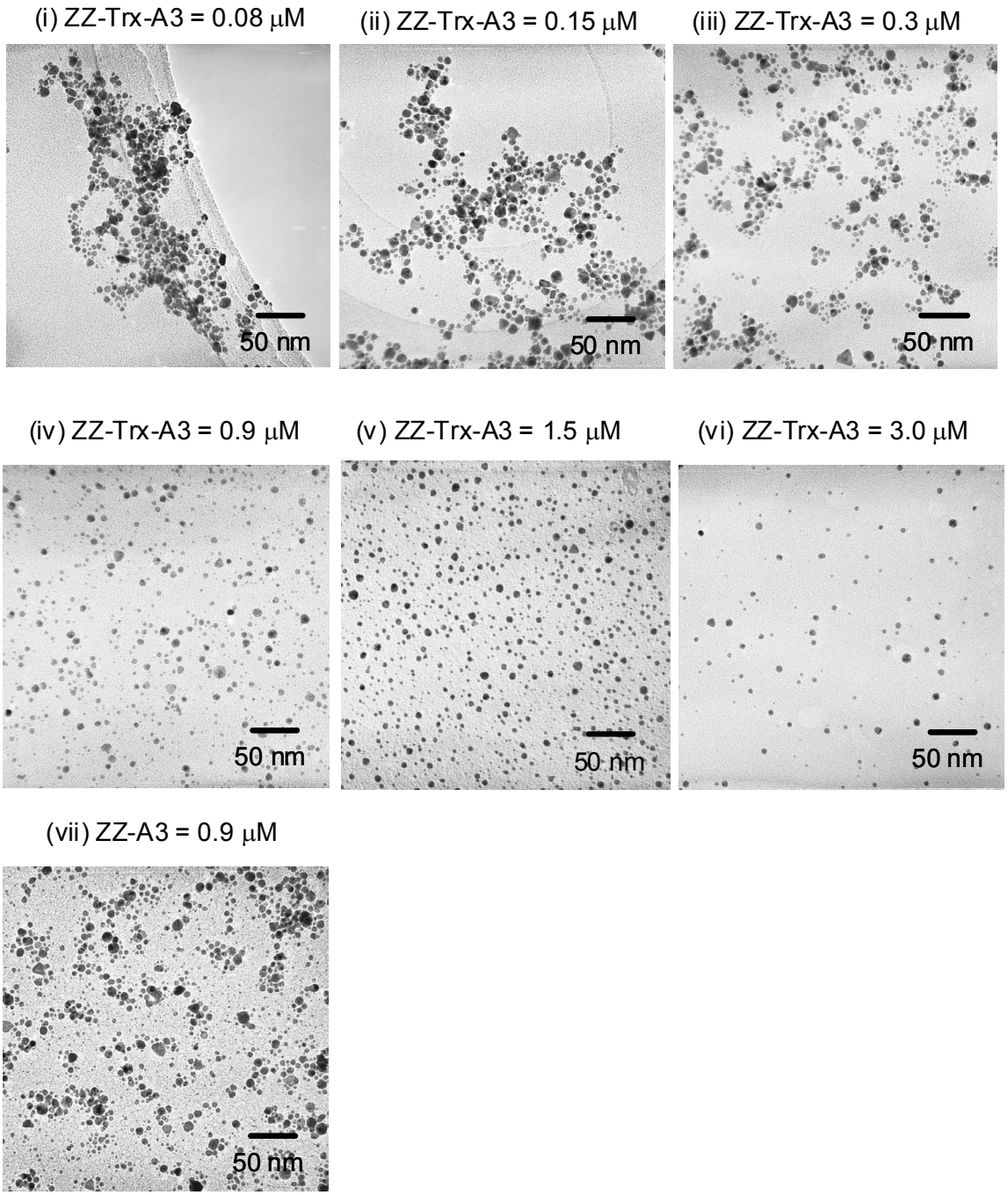


Fig. S6 TEM images of the AuNPs synthesized by varying the ZZ-Trx-A3 concentration. The samples obtained in Fig. S5 were employed for the TEM measurements. Image (v) is identical to Fig. 1 (d) in the main text. Sample (vii) is the AuNPs synthesized using the ZZ-A3 protein. [HEPES] = 10 mM, pH 4.0; [$HAuCl_4$] = 0.25 mM.

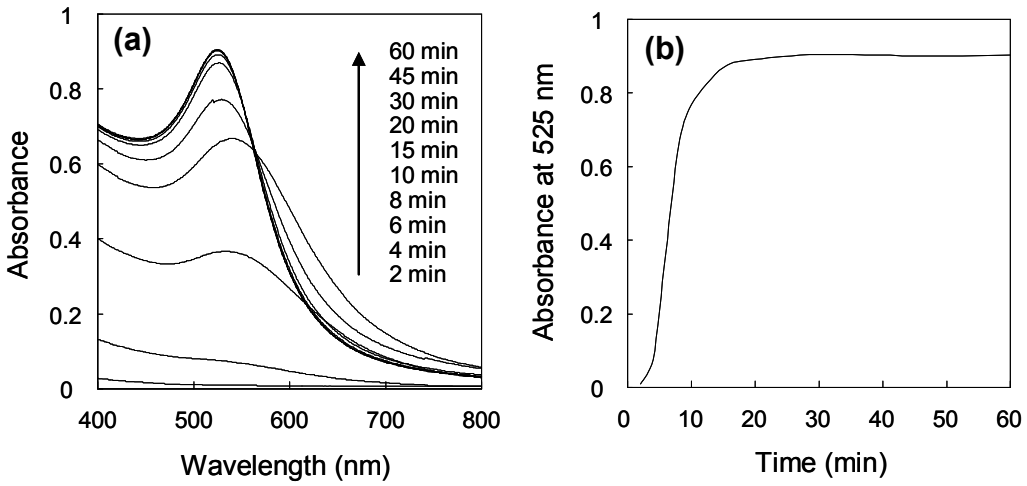


Fig. S7 Time course of AuNPs biofabrication using the ZZ-Trx-A3 protein. (a) UV/Vis spectra and (b) absorbance at 525 nm. [ZZ-Trx-A3] = 1.5 μM; [HEPES] = 10 mM, pH 4.0; [$HAuCl_4$] = 0.25 mM.

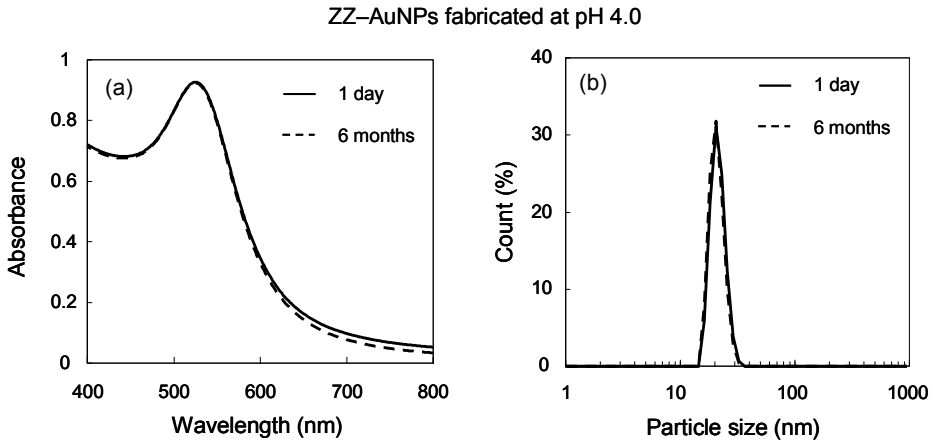


Fig. S8 Long-term stability of ZZ–AuNPs fabricated at pH 4.0. (a) UV/Vis spectra, (b) DLS data. The ZZ–AuNP solutions were stored at 4°C for over 6 months.

4. ELISA procedure using the AuNP–ZZ domain conjugates

The ZZ–AuNPs were synthesized under the optimal aqueous condition (10 mM HEPES at pH 4.0, 1.5 μM ZZ-Trx-A3). After incubation for 2 h at room temperature, the ZZ–AuNP solution (1.8 mL) was adjusted to pH 8.0 by adding 0.45 mL of 100 mM Tris buffer solution (pH 8.6) and this solution was stored at 4 °C prior to use. The aqueous solution of BSA (1 mg/mL) was prepared with TBS (50 mM Tris-HCl (pH 7.6), 150 mM NaCl). One hundred microliters of the BSA solution was added to each well of a 96-well immuno plate. After incubation for 1 h at room temperature, the wells were washed three times with 200 μL of TBST (TBS supplemented with 0.05 % Tween-20). To block nonspecific binding sites, the wells were incubated with 300 μL of a casein solution (10 mg/mL, TBS) for 1 h at room temperature. After three cycles of washing, 100 μL of a 10,000-fold dilution of rabbit anti-BSA IgG (ROCKLAND) was added to the wells. The plate was incubated for 1 h at room temperature and washed three times with 200 μL of TBST. A 100 μL aliquot of the neutralized ZZ–AuNP solution was added to the wells. After incubation for 1 h at room temperature, the wells were washed as described above. One hundred microliters of a 500-fold dilution of HRP-labeled antibody to goat IgG produced in rabbit (KPL) was added to each well. The plate was incubated for 1 h at room temperature and washed three times with 200 μL of TBS. The wells were supplied with 200 μL of the substrate solution freshly prepared by dissolving 4 mg of 1,2-phenylenediamine in 10 mL of 100 mM Tris buffer (pH 7.0), supplemented with 5 μL of H_2O_2. The reaction was followed spectroscopically by monitoring the absorbance at 415 nm on a spectral scanning multimode reader Varioskan Flash 2.4 (Thermo Fisher Scientific) for 90 min.

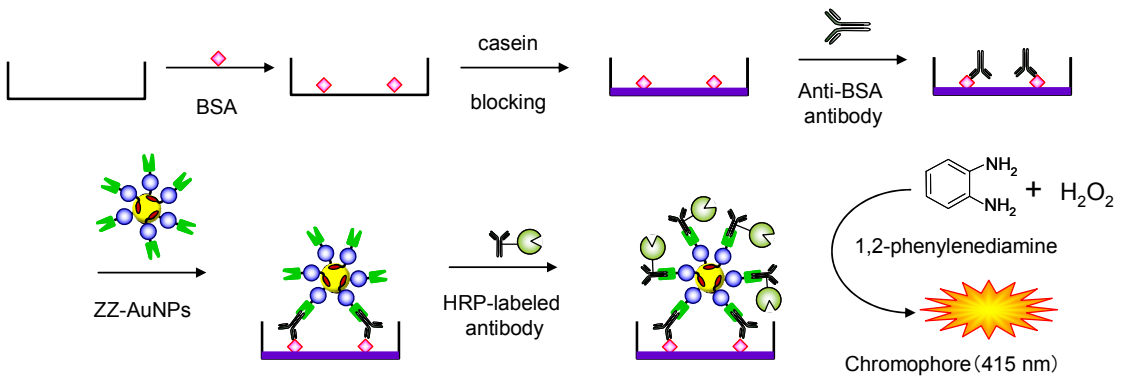


Fig. S9 Schematic illustration of the ELISA in this study.

5. Immunoassay based on the antigen-responsive AuNPs aggregation

The ZZ–AuNPs were synthesized under the optimal aqueous condition and adjusted to pH 8.0 according to the procedure described above. One milliliter of the neutralized ZZ–AuNP solution was mixed with 13 μL of the rabbit anti-lysozyme IgG (NORDIC, 13.8 mg/mL: 94 μM); equivalent to the amount of the ZZ-Trx-A3 protein used for mineralization. The mixture was incubated for 2 h at room temperature with gentle mixing. A 6 μL aliquot of a lysozyme solution (1.35 mg/mL: 94 μM, TBS) was added, and the mixture was incubated for 2 h at room temperature with gentle mixing. The aggregation reaction was characterized by DLS, UV/Vis and TEM measurements. The control experiment was carried out using BSA instead of lysozyme. In addition, the comparison experiment was conducted using AuNPs fabricated using a pH 8.0 HEPES buffer solution (Fig. S12).

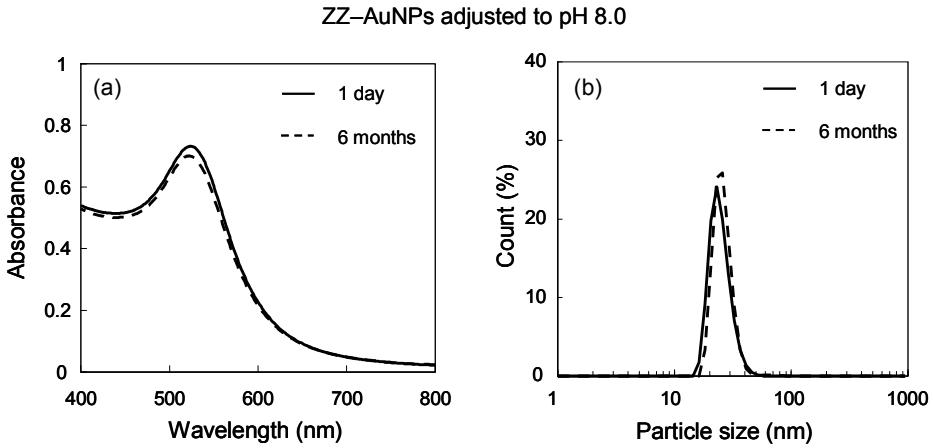


Fig. S10 Long-term stability of ZZ–AuNPs adjusted to pH 8.0. (a) UV/Vis spectra, (b) DLS data. The ZZ–AuNP solutions were stored at 4°C for over 6 months.

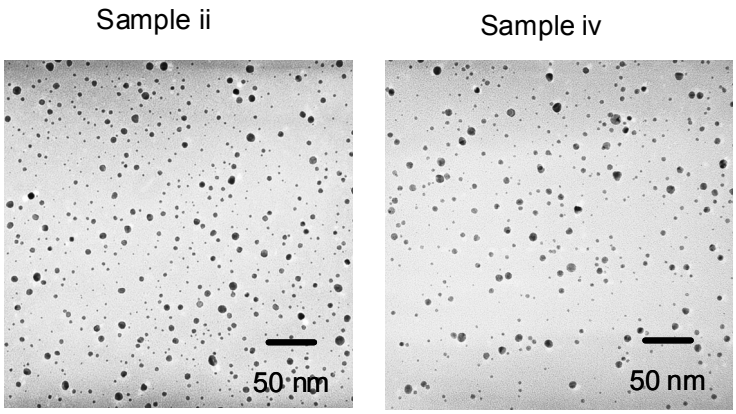
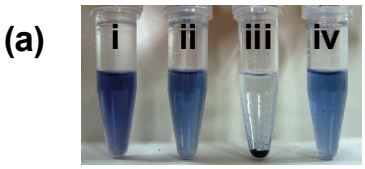


Fig. S11 TEM images of the AuNPs (samples ii and iv) mentioned in Fig. 3. Sample ii: ZZ–AuNPs + anti-lysozyme IgG; sample iv : ZZ–AuNPs + anti-lysozyme IgG + BSA.

Sample	i	ii	iii	iv
ZZ-AuNPs (pH 8.0)	+	+	+	+
Anti-lysozyme IgG	–	+	+	+
Lysozyme	–	–	+	–
BSA	–	–	–	+



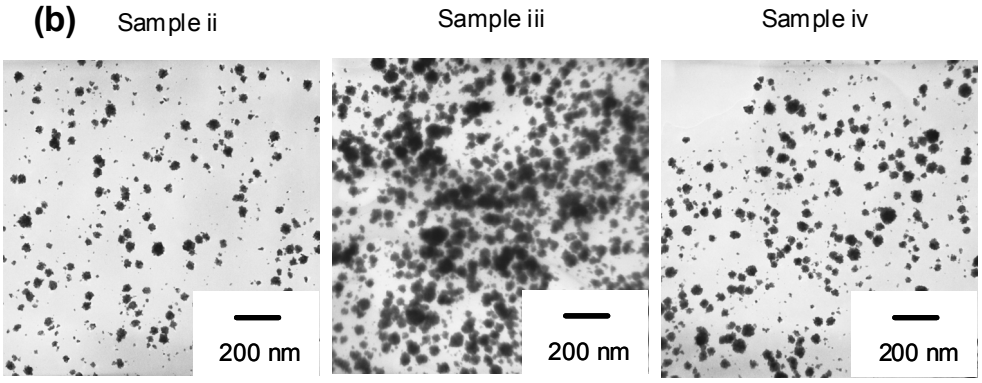


Fig. S12 Antigen-responsive aggregation using the AuNPs synthesized at pH 8.0. (a) Appearances of corresponding samples and (b) TEM images of the AuNPs (samples ii, iii and iv).