Electronic Supplementary Information

Preparation and catalytic reaction of Au/Pd bimetallic nanoparticles in Apo-Ferritin

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Materials. Reagents were purchased from TCI, Wako, Nacalai Tesque, and Sigma-Aldrich and used without further purification. Recombinant L-chain apo-Fr from horse liver was prepared in NovaBlue competent cells (Novgen) transformed with the expression vector pMK2 kindly supplied by Prof. Ichiro Yamashita. The culture and purification of the protein was performed according to a previous report[1]. **(Pd-NP)•apo-rHLFr** was prepared by the reported method[2]. Metal ion content in apo-rHLFr was determined by Inductively coupled plasma-optical emission spectrometry (ICP-OES) and Bicinchoninic Acid (BCA) protein assays.

Preparation of Au^{3+}•apo-rHLFr. An apo-rHLFr solution (1 μM, 30 mL) in 0.15 M NaCl aqueous solution was adjusted to pH 8.5 with 0.01 M NaOH, followed by the addition of aliquots of $KAuCl_4$ aqueous solution (100 equiv.). After stirring for 30 min, the solution was concentrated to approximately 10 mL and subsequently purified using a gel filtration column (Superdex G-200) equilibrated with 0.15 M NaCl aqueous solution**.**

Crystallization of Au^{3+}•apo-rHLFr and the data collection. Crystallization was performed using the hanging drop vapor diffusion method as described in a previous report.[3] Purified **Au^{3+}•apo-rHLFr** solution was concentrated to 10-20 gL^{-1} and drops were produced by mixing an equal volume (1 μL) of the protein solution (20 mM Tris/HCl, 0.15 M NaCl aq.) and the precipitant solution (0.5-1 M $(NH_4)_2SO_4$, 20-30 mM $CdSO_4$), and equilibrated against the precipitant solution (1 mL) at 20℃. Before data collection, single crystals were immersed in a precipitant solution containing 30 % (w/w) glycerol and subsequently frozen in liquid nitrogen. X-ray diffraction data of **Au^{3+}•apo-rHLFr** were collected at 100K at beamLine BL38B1 at SPring-8 using X-ray

wavelengths of 1.0395 Å, and 1.0578 Å which represent the peak and remote wavelengths of Au X-ray absorption, respectively. Crystallographic data are summarized in Table S1. Atomic coordinate is deposited in the Protein Data Bank under accession ID: 3H7G.

Structure analysis. Data were processed with the program *HKL2000* in the cubic *F*432 space group. The structures were solved by molecular replacement with *MOLREP* using apo-rHLFr structure (pdb code: 1DAT) as an initial model. Refinement of the protein structure was performed using the program *REFMAC5* with higher resolution data of **Au^{3+}•apo-rHLFr** were collected at 1.0395 Å. Model biases were reduced by composite omit maps calculated with *CNS*.[4] Rebuilding was carried with *COOT*. The solvent was identified to fit residual (F_o-F_c) density peaks with a lower cut-off of 3 σ. Refinement statistics are summarized in Table S1.

Anomalous analysis. Metal ions in **Au^{3+}•apo-rHLFr** crystals were identified using the differences in anomalous scattering effect of two wavelengths as reported[5]. The data were collected at 1.0395 Å, for the Au peak wavelength. The other data set were collected at 1.0578 Å, for the Au Remote wavelength. Anomalous difference Fourier maps were calculated for all data sets at 1.70 Å, and the coordination structures of the Au and Cd binding sites were picked up at 4 σ.

Preparation of (Pd-NP)•apo-rHLFr . An apo-rHLFr solution (1 μM, 10 mL, in 150 mM NaCl) was adjusted to pH 8.5 with 0.01 N NaOH, followed by the addition of aliquots of a $KPdCl_4$ aqueous solution (500 equiv., 40 mM). The mixture was stirred for 30 min, followed by the addition of a $NaBH_4$ aqueous solution (2500 equiv. for **(Pd-NP)•apo-rHLFr**). After stirring for 30 min, the solution was dialyzed against 5 L of a 150 mM NaCl aqueous solution for 12 h and finally purified by gel filtration (Superdex G-200).

Preparation of (AuPd-NP) •apo-rHLFr. An apo-rHLFr solution (1 μM, 20mL, in 150 mM NaCl) was adjusted to pH 8.5 with 0.01 M NaOH, followed by the addition of aliquots of a $KAuCl_4$ aqueous solution (500 equiv.). The mixture was stirred for 30 min, then, filtrated by gel chromatography (Superdex G-200) to remove Au^{3+} ions

remaining the solution. The protein concentration of the eluate was determined by the BCA method. To the eluate, aliquots of a K_2PdCl_4 aqueous solution (500 equiv.) was added. The mixture was stirred for 30 min, followed by the addition of a $NaBH_4$ aqueous solution (5000 equiv.). After stirring the mixture for 30 min, the sample was dialyzed against 5L of a 150 mM NaCl aqueous solution for 12 h. Finally the sample was purified by gel filtration (Superdex G-200).

Preparation of ([Au](Pd)-NP)•apo-rHLFr. The Au core particle was prepared by the same procedure reported previously.[2] A purified **(Au-NP)•apo-rHLFr** solution was adjusted pH 8.5 with 0.01 M NaOH and aliquots of a K_2PdCl_4 aqueous solution (500 equiv.) was added. After the stirring of the mixture for 30 min, a $NaBH_4$ aqueous solution (2500 equiv.) was added and stirred for another 30 min, followed by dialysis and gel filtration.

Preparation of ([Pd](Au)-NP)•apo-Fr. The Pd core particle was prepared according to by the procedure of Pd•apo-rHLFr using K_2PdCl_4 (100 equiv) and $NaBH_4$ (500 equiv.). After the adjustment of a purified Pd•apo-rHLFr solution at pH 8.5 with 0.01 N NaOH, an aqueous solution of $KAuCl_4$ (500 equiv.) was added and stirred for 30 min. The resulting solution was treated with a $NaBH_4$ aqueous solution (2500 equiv.) and stirred for 30 min, and the composite was purified by dialysis and gel filtration.

Hydrogenation reaction. Hydrogenation reactions were carried out at 7 °C under a H_2 atmosphere with a flow rate of 50 $mLmin^{-1}$ as reported before.[2] A D_2O solution containing acrylamide (1 mL) was bubbled with H_2 for 15 min, then, the hydrogenation was initiated by the addition of 1 mL of **(AuPd-NP)•apo-rHLFr**, **([Au](Pd)-NP)•apo-rHLFr** (final concentration of total metal atoms in the solution is *ca.* 6 μM). The products were identified by ^1H NMR measurements of the reaction mixtures. The TOF of **(Pd-NP)•apo-rHLFr** for acrylamide is remarkably higher than that reported before.[2] It is because the current samples were prepared and stored under Ar atmosphere to prevent air oxidation of Pd-NPs while **(Pd-NP)•apo-rHLFr** used before was stored under air. Thus, all the samples of **(AuPd-NP)•apo-rHLFr** and **([Au](Pd)-NP)•apo-rHLFr** are also stored under Ar.

Physical Measurements. Absorption spectra were recorded on a Shimazu UV-2400PC UV–vis spectrometer. Metal concentrations were determined by using an inductively coupled plasma atomic emission (ICP) spectrometer (Varian ICP-OES Vista-PRO). $PdCl_2$ in 0.1 mol/L•HCl (1.01 mg/mL) and $HAuCl_4$ in 0.1 mol/L•HCl (1.00 mg/mL) were used as calibration standards and $Y(NO_3)_3$ in 0.1 mol/L•HNO_3 (1.01 mg/L) was applied for an internal standard. Transmission electron micrographs (TEM) and high-resolution TEM (HRTEM) were obtained by using a JEOL-2010HC microscope equipped with a CT-3500 cryo-holder and a JEM-3000F microscope having a point-to-point resolution of 0.17 nm, respectively. Samples for TEM and HRTEM measurement were prepared by depositing a drop of a sample solution on a carbon-coated Cu grid (QUANTIFOIL R2/2 purchased from Quantifoil Micro Tools GmbH for TEM and Micro grid A type purchased from Okenshoji Co., Ltd. for HRTEM). In the case of TEM samples, a sample grid was rapidly plunged into liquid propane (–190 °C) for cryo observation. Single-particle EDS analysis was carried out using a VANTAGE digital microanalysis system. The data were collected using a take off angle of 20 °, an acceleration voltage of 300 kV, and a collection time of 100s. ^{1}H NMR spectra were recorded on a JEOL JNM-A 400 NMR spectrometer.

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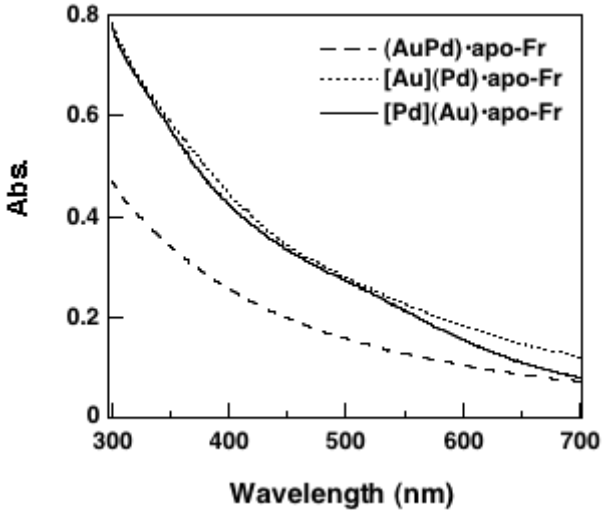

Fig. S1. UV-vis spectra of (AuPd)•apo-rHLFr, [Au])(Pd)•apo-rHLFr, and [Pd](Au)•apo-rHLFr in 0.15 M NaCl at pH 6.

Table S1. Summary of X-ray Data and Refinement Statistics for **Au^{3+}•apo-Fr**.

	Au^{3+}•apo-rHLFr	
	Au Peak	Au Remote
Data collection statistics		
X-ray source	BL38B1	BL38B1
X-ray wavelength	1.0395	1.0578
Space group	*F432*	*F432*
Unit cell		
$a = b = c$ (Å)	181.632	181.874
Molecular per symmetric unit	24	24
Resolution range	35.0-1.65	35.0-1.70
Total observations	634758	592417
Unique reflections	31508	28909
Completeness (%)[a]	100.0 (100.0)	100.0 (100.0)
R_{merge} (%)[a,b]	8.2 (30.9)	7.3 (29.8)
I/σ (I) [a]	67.8 (8.01)	71.2 (9.03)
Refinement statistics		
Resolution (Å)	30.3-1.65	
No. reflections Working set/test	29893/1590	
R-facror (%)[c]	21.0	
R_{free} (%)[d]	23.6	
Final model		
No. of residues	173 (2-174)	
No. of water molecules	177	
No. of sulfate ions	3	
No. of glycerol molecules	4	
No. of Pd ions	0	
No. of Au ions	6	
No. of Cd ions	3	
rms diviation from ideality		
Bonds (Å)	0.008	
Angle (°)	1.105	
Ramachandran plot (%)[e]		
most favored	94.3	
allowed	5.7	

[a] Values in parentheses are for the highest resolution shell. [b] $R_{merge} = \sum|I-\langle I\rangle|/\sum I$,

where I is the integrated intensity of a given reflection. [c] $R = \sum||F_O|-|F_C||/\sum|F_O|$, where F_O and F_C are the observed and calculated structure factor amplitudes, respectively. [d] Rfree: an R factor calculated on a partial set that is not used in the refinement of the structure. [e] Ramachandran plot parameters were calculated using *PROCHECK*.[6]