Electronic Supplementary Information

Definite Coordination Arrangement of Organometallic Palladium Complexes on the Designed Interior Surface of Apo-ferritin

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Experiment Section

Materials. Reagents were purchased from TCI, Wako, Nacalai Tesque, and Sigma-Aldrich and used without further purification. Apo recombinant L-chian ferritin from horse liver (apo-rHLFr) was prepared in NovaBlue competent cells (Novagen) transformed with the expression vector pMK2. Apo-rHLFr mutants, **apo-E45C/C48A-rHLFr**, **apo-E48C/R52H-rHLFr** and **apo-E45C/H49A/R52H-rHLFr** were prepared using QuikChange Site-Directed Mutagenesis Kit (Stratagene). The culture and purification of the proteins were performed according to a previous literature.[1] The preparation of Pd(allyl)·apo-rHLFr mutants and the evaluation of their catalytic reactivity were done by the methods reported previously.[2]

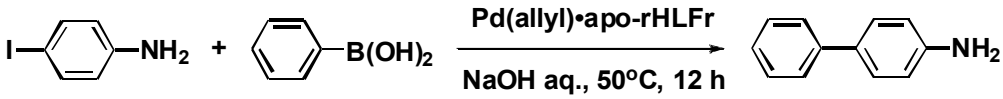
Preparation of Pd(allyl)·apo-rHLFr Mutants. Pd(allyl)·apo-E45C/C48A-rHLFr, **Pd(allyl)·apo-E48C/R52H-rHLFr** and **Pd(allyl)·apo-E45C/H49A/R52H-rHLFr** were prepared by the process described below: 500 μL $[Pd(allyl)Cl]_2$ solution (20 mM in acetonitrile) was added into 5 mL apo-rHLFrs solution (10 μM in the buffer of Tris/HCl (50 mM, pH = 8)). The mixture was stirred for 1 hour at room temperature and then dialyzed against 2 L NaCl solution (0.15 M) at 4 °C overnight. The product Pd(allyl)·apo-rHLFr mutants were obtained after purifying the reaction mixture with Superdex G200 size-exclusion chromatography using NaCl solution (0.15 M) as the eluent. The number of Pd atoms in Pd(allyl)·apo-rHLFr mutants were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) and bicinchoninate (BCA) method.

Crystallization of Pd(allyl)·apo-rHLFr Mutants. Hanging drop vapor diffusion method as described in previous reports[2-4] was used to crystallize Pd(allyl)·apo-rHLFr mutants. The solutions of Pd(allyl)·apo-rHLFr mutants were concentrated to about 30 mg/mL in NaCl solution (0.15 M). The drops were prepared by mixing an equal volume (3 μL) of the protein solution and the precipitant solution (1 M $(NH_4)_2SO_4$, 15-20 mM $CdSO_4$), and then equilibrated against the same precipitant solution (1 mL) at 20 °C. The crystals were produced within one day.

X-ray Crystallography and Data Refinement. After crystallization, single crystals of Pd(allyl)·apo-rHLFr mutants were immersed in a precipitant solution containing 30% (w/w) ethylene glycol and subsequently frozen in liquid nitrogen. X-ray diffraction data of Pd(allyl)·apo-rHLFr mutants were collected at 100 K at beamline BL38B1 of SPring-8 using X-ray with wavelength of 1.0 Å. The data was processed using HKL2000 programs as cubic *F432* space group. Refinement of the protein structure was performed using *REFMAC5*[5] of the *CCP4* suite and rebuilding was performed using *COOT*[6] based on sigma weighted ($2F_o$-F_c) and (F_o-F_c) electron density maps. The crystal parameters, data collection statistics and refinement statistics are summarized in Table S1. Atomic coordinates are deposited in the Protein Data Bank under accession numbers 3NP2, 3NOZ, and 3NP0 for **Pd(allyl)·apo-E45C/C48A-rHLFr**, **Pd(allyl)·apo-E45C/R52H-rHLFr** and **Pd(allyl)·apo-E45C/H49A/R52H-rHLFr**, respectively. Residue Ser1 and Asp174 in **Pd(allyl)·apo- E45C/C48A-rHLFr** were not decided because of their disordered electron densities. Residue Glu130 and Gln158 in **Pd(allyl)·apo-E45C/C48A-rHLFr**, Asp127, Glu130, Ser131 and His132 in **Pd(allyl)·apo -E45C/R52H-rHLFr**, and His49, Asp127, Glu130, Ser131, His132, Glu136, Lys172 and

Asp174 in **Pd(allyl)·apo-E45C/H49A/R52H-rHLFr** were replaced to Ala because electron densities of these residues are missing.

Suzuki Coupling Reaction.



The reactions were performed in an aqueous solution of 0.15 M NaCl which contained *p*-I-PhNH$_2$ (2.5 mM) and PhB(OH)$_2$ (5.2 mM) as substrates, NaOH (4.5 mM) as the base and Pd(allyl)·apo-rHLFr mutant (0.025 μM) as catalyst. After incubation at 50 °C for 12 h, the reactions were terminated by adding HCl to pH 1.9. The mixtures were extracted with chloroform and then the solvent was removed by vacuum evaporation. By analysis with ^{1}H-NMR spectroscopy, the turnover frequencies (TOF = [product (mol)] per Pd(allyl)·apo -rHLFr per hour) of the coupling reactions were calculated based on the consumption of *p*-I-PhNH$_2$ and the formation of the product.

Reference

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Table S1. Summary of X-ray Data and Refinement Statistics for **Pd(allyl)·apo-rHLFr Mutants.**

	Pd(allyl)·apo-E45C/C48A-rHLFr	**Pd(allyl)·apo-E45C/R52H-rHLFr**	**Pd(allyl)·apo-E45C/H49A/R52H-rHLFr**
Data collection statistics			
X-ray wavelength, Å	1.0000	1.0000	1.0000
Space group	*F432*	*F432*	*F432*
Unit cell, Å	182.296	181.427	180.959
Resolution range (outer shell) (Å)	30-1.86 (1.93-1.86)	25-1.52 (1.57-1.52)	30-1.48 (1.53-1.48)
Total observations	423,914	751,588	765,474
Unique reflections	40,701	73,696	78,341
Completeness (%)[a]	98.6 (100)	99.2 (93.9)	98.3 (88.7)
R_{merge} (%)[a,b]	12.7 (28.5)	5.4 (34.7)	6.4 (28.6)
$I/\sigma(I)$ [a]	65.9 (19.5)	59.9 (5.3)	66.6 (7.3)
Refinement statistics			
Resolution (Å)	24.36-1.86	19.34-1.52	19.29-1.48
No. reflections Working set/test	19,613/1,049	37,012/1,951	39,184/2,070
R-factor (%)[c]	19.9	19.5	19.8
R_{free} (%)[d]	22.8	20.8	21.0
Final model			
No. of residues	172 (2-173)	174 (1-174)	174 (1-174)
No. of water molecules	165	173	211
No. of Pd complexes and Pd atoms	4	6	6
No. of Cd atoms	2	1	1
No. of Sulfate ions	1	1	2
No. of ethylene glycol	8	9	8
rms diviation from ideality			
Bonds (Å)	0.018	0.016	0.012
Angle (°)	1.519	1.598	1.613
Ramachandran plot (%)[e]			
most favored	93.6	91.8	91.8
allowed	6.4	8.2	8.2

[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] R_{free}: 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*.

Table S2. Selected Distances of Pd–S(Cys), Pd–N$^{\delta}$(His), Pd–O(H_2O), and Pd-Pd in the accumulation centers of **Pd(allyl)·apo-rHLFr Mutants**.

	Pd–X distances (Å)
Pd(allyl)·apo-E45C/C48A-rHLFr	
PdB1–S$^{\gamma}$(Cys45)	2.40
PdB1–N$^{\varepsilon}$(His173)	2.98
PdB2–S$^{\gamma}$(Cys45)	2.27
PdB2–N$^{\varepsilon}$(His49)	1.92
PdB1–PdB2	3.21
Pd(allyl)·apo-E45C/R52H-rHLFr	
PdC1–S$^{\gamma}$(Cys45)	2.26
PdC1–N$^{\varepsilon}$(His173)	2.22
PdC2–S$^{\gamma}$(Cys45)	2.39
PdC2–N$^{\varepsilon}$(His49)	2.04
PdC1–PdC2	3.19
PdC3–S$^{\gamma}$(Cys48)	2.41
PdC3–N$^{\delta}$(His52)	2.12
PdC4–S$^{\gamma}$(Cys48)	2.36
PdC4–O(WC1)	2.27
PdC3–PdC4	3.32
Pd(allyl)·apo-E45C/H49A/R52H-rHLFr	
PdD1–S$^{\gamma}$(Cys45)	2.38
PdD1–N$^{\delta}$(His173)	2.29
PdD2–S$^{\gamma}$(Cys45)	2.32
PdD2–O(WD1)	2.35
PdD1–PdD2	3.44
PdD3–S$^{\gamma}$(Cys48)	2.37
PdD3–N$^{\delta}$(His52)	2.21
PdD4–S$^{\gamma}$(Cys48)	2.44
PdD4–O(WD2)	2.36
PdD3–PdD4	3.21
Pd(allyl)·apo-rHLFr[a]	
PdA1–S$^{\gamma}$(Cys48)	2.57
PdA1–N$^{\delta}$(His49)	2.17
PdA2–S$^{\gamma}$(Cys48)	2.31
PdA2–O$^{\varepsilon}$(Glu45)	2.30
PdA1–PdA2	3.12

a The structure was taken from PDB (PDB ID: 2ZG7).

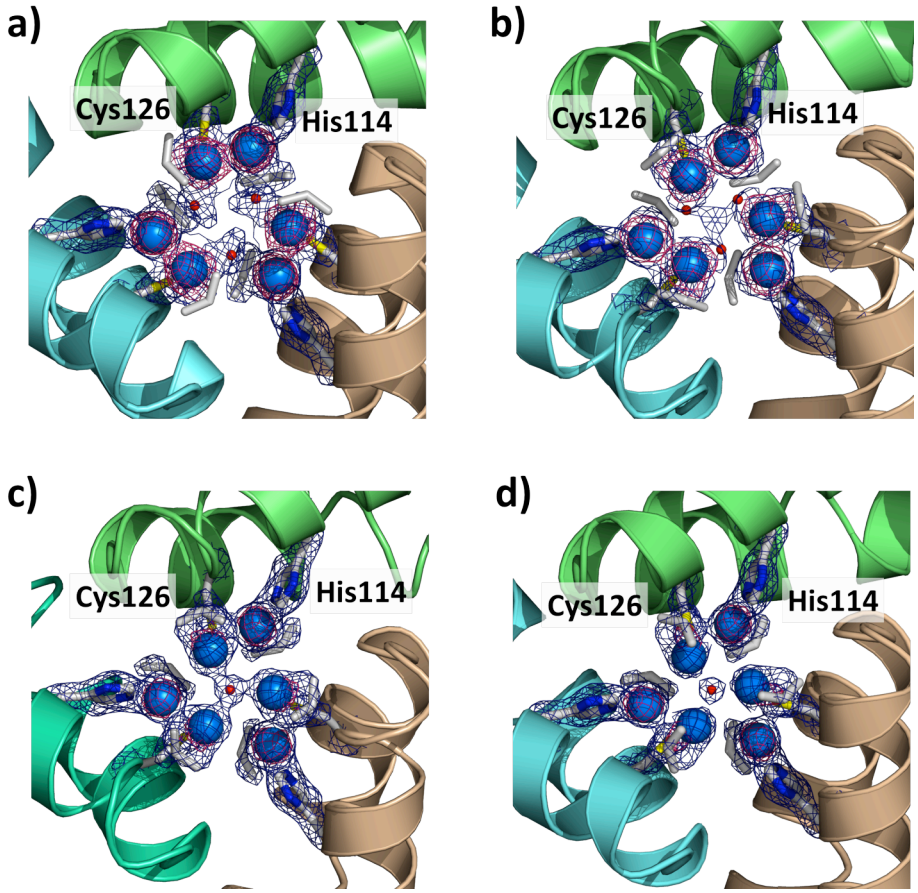

Fig. S1. The 3-fold channels of **Pd(allyl)·apo-rHLFr** (a), **Pd(allyl)·apo-E45C/C48A -rHLFr** (b), **Pd(allyl)·apo-E45C/R52H-rHLFr** (c), and **Pd(allyl)·apo-E45C/H49A/ R52H-rHLFr** (d). The Pd atoms are indicated as sphere models colored in cyan. The O atoms of water molecules are shown as red spheres. The anomalous difference Fourier maps at 4.0 σ indicate the positions of palladium atoms that are shown in magenta. The selected $2|Fo| - |Fc|$ electron density maps at 1.0 σ are shown in blue.

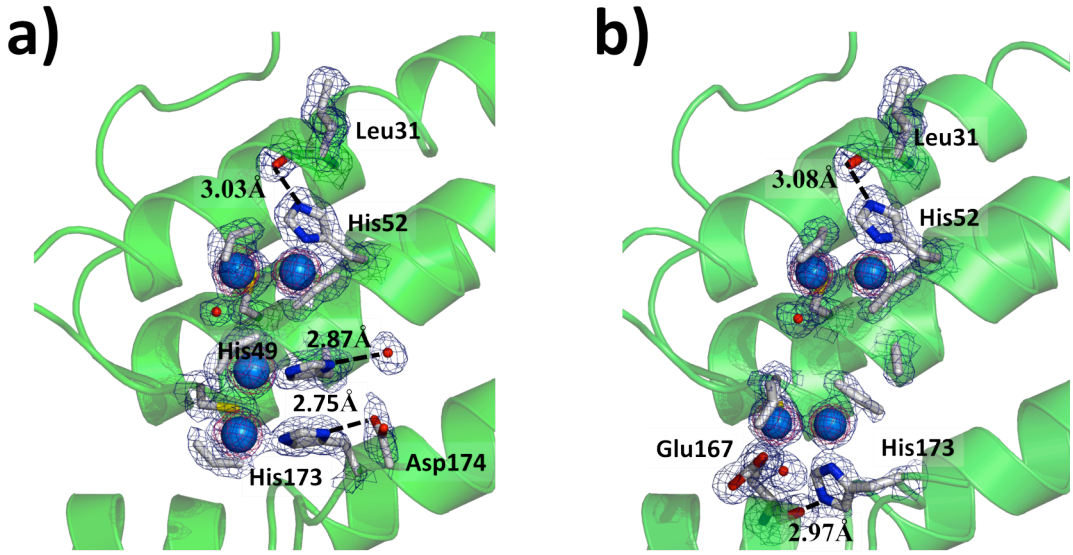

Fig. S2. Hydrongen bonds of His49, His52, and His173 in **Pd(allyl)·apo-E45C/R52H -rHLFr** (a), and hydrogen bonds of His52 and His173 **Pd(allyl)·apo-E45C/H49A/R52H -rHLFr** (b).