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Structure of in cell protein crystals containing organometallic complexes

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General Information

Materials

Reagents were purchased from TCI, Wako, Nacalai Tesque, and Sigma–Aldrich were used without further purification. The *Spodoptera frugiperda* cell line IPLB–Sf21–AE (Sf21) was maintained in tissue culture flasks in Grace's medium (Gibco–BRL) at 27˚C with 10% fetal bovine serum (MP Biomedicals. Inc.), 2.6 mg/mL tryptose broth, 100 U/mL penicillin, and 100 μg/mL streptomycin.

Equipments

SEM analyses were performed on FE-SEM, Hitachi S-5500 after treatment of sample with platinum coater (Hitachi, MC1000). Elemental mapping of Pd was investigated using energy dispersive X-ray spectrum (EDX).

Preparation of WTPhC and ΔL4-PhC. Expression of recombinant wild-type *Bombyx mori* CPV polyhedra (WTPhC) and ΔL4-PhC in insect cells and purification of WTPhC were performed according to a previous literature.[1, 2]

Preparation of Pd(allyl)•WTPhC and Pd(allyl)•ΔL4-PhC. WTPhC or ΔL4-PhC (4.0×10^7 crystals) were soaked in acetonitrile solution containing $[Pd(allyl)Cl]_2$ (50 mM) for 24 h at 37 °C. The numbers of the crystals were calculated with a Bürker-Türk counting chamber. The composites were then washed with water to remove excess Pd(allyl) complexes.

SEM analysis.

SEM analyses of **Pd(allyl)•WTPhC** and **Pd(allyl)•ΔL4-PhC** were performed on field-emission scanning electron microscopy (FE-SEM, Hitachi S-5500) after treatment of sample with platinum coater (Hitachi, MC1000). Elemental mapping of Pd in **Pd(allyl)•WTPhC** and **Pd(allyl)•ΔL4-PhC** was investigated using energy dispersive X-ray spectrum (EDX).

X-ray crystal structure analysis of Pd(allyl)•WTPhC and **Pd(allyl)•ΔL4-PhC.** Prior to the data collection, **Pd(allyl)•WTPhC** and **Pd(allyl)•ΔL4-PhC** were immersed in a buffer solution containing 50 % (w/w) ethylene glycol and were spread on MicroMesh and subsequently frozen in liquid nitrogen. X-ray diffraction data of **Pd(allyl)•WTPhC** and **Pd(allyl)•ΔL4-PhC** were collected at 100 K at

beamline BL32XU at SPring-8 using X-ray wavelength of 1.00 Å. The complete sets of structure factor amplitudes were obtained by merging multiple small-wedge (5° or 10° each) datasets collected from single crystals. The crystal positions in a cryoloop were identified by low-dose raster scan. The whole data collection process was automated by ZOO system including sample exchange by a robot. Collected datasets were automatically processed and merged by KAMO.[3] Each dataset was indexed and integrated using XDS.[4] The datasets consistently indexed with the known cell parameter (a ~ 103 Å, I23) were selected and two possible reindex operators (*hkl* and -*hlk*) were tested to give better match to the previously solved data (2OH6). The datasets were subjected to hierarchical clustering by pairwise correlation coefficient of intensities. The datasets in each cluster were scaled and merged using XSCALE[4] with outlier rejections implemented in KAMO. The clusters with the highest $CC_{1/2}$ were chosen for downstream analyses. The structure was solved by rigid body refinement with phenix.refine[5] using the previously solved structure (2OH6). Refinement of the protein structure was performed at resolutions of 1.58 and 2.08 Å for **Pd(allyl)•WTPhC** and **Pd(allyl)•ΔL4-PhC**, respectively, using REFMAC5[6] in the CCP4 suite. Rebuilding was performed using COOT[7] based on sigma-A weighted (2*Fo-Fc)* and (*Fo-Fc*) electron density maps. N-terminal region (ACE1- Arg10), L2, H3 (Leu68-Ser102), L3 (Ala129-Asp134) and L4, H4 (Pro186-Cys204) in **Pd(allyl)•ΔL4-PhC** could not be modeled because electron densities corresponding to these residues are missing. The positions of Pd atoms were determined from the Fourier difference and anomalous electron density maps. The occupancy values of Pd atoms were adjusted manually by considering negative density and refined with fractional occupancy. The models were subjected to quality analysis during the various refinement stages with omit maps and RAMPAGE.[8] The diffraction and refinement statistics are summarized in Table S1 and S2, respectively.

Accession Codes: Atomic coordinates for **Pd(allyl)•WTPhC** and **Pd(allyl)•ΔL4-PhC** have been deposited in the Protein Data Bank under accession codes 5YHA and 5YHB, respectively.

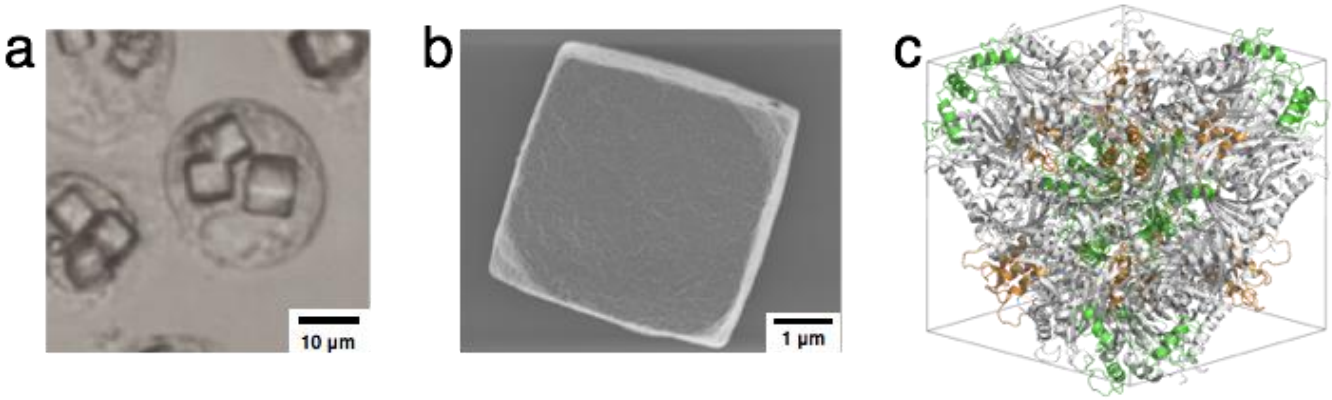

Figure S1. (a) Recombinant WTPhC in Sf21 insect cells. (b) SEM image of recombinant of WTPhC. (c) Molecular assembly structure of WTPhC taken from PDB ID :2OH6.

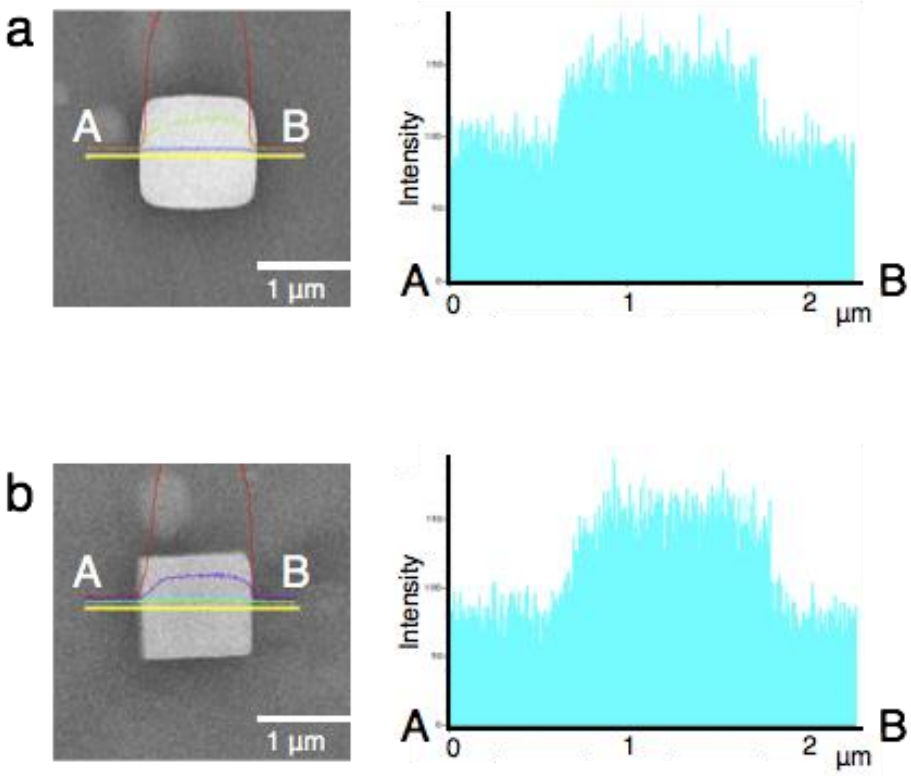

Figure S2. SEM-EDX images and the line-scan EDX intensity profiles of (a) **Pd(allyl)•WTPhC** and (c) **Pd(allyl)• ΔL4-PhC**.

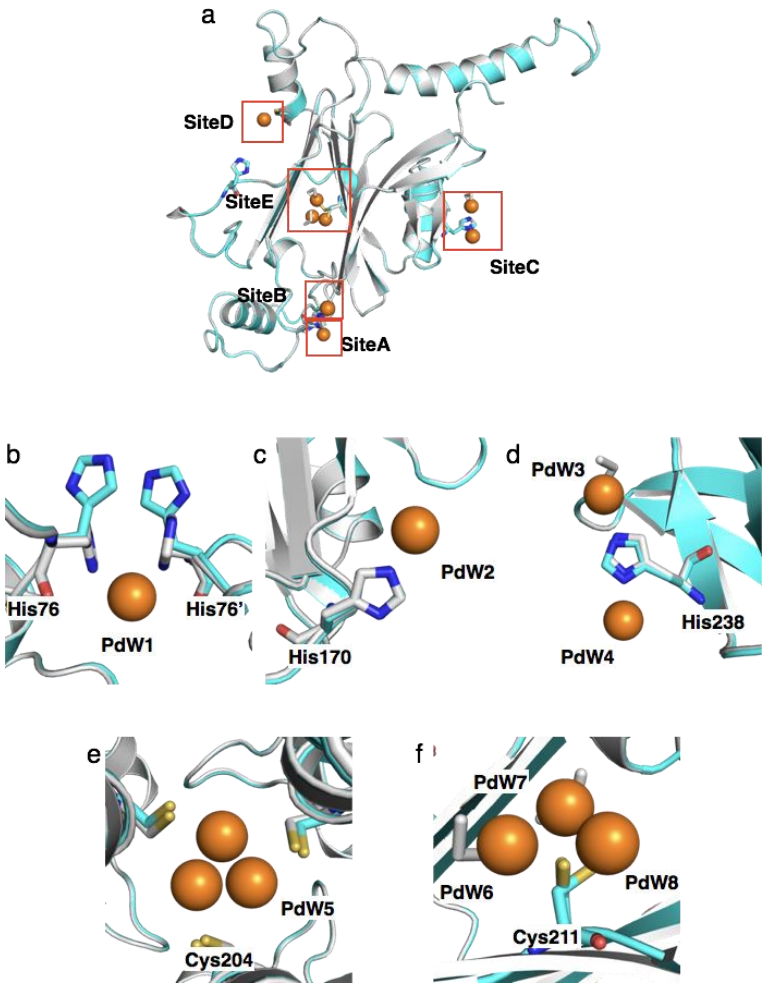

Figure S3. Superimposed structures of **Pd(allyl)•WTPhC** (white) and WTPhC (cyan). (a) Monomer structures and metal binding structures of (b) Site A, (c) Site B, (d) Site C, (e) Site D and (f) Site E. The Pd atoms are indicated by orange spherical models.

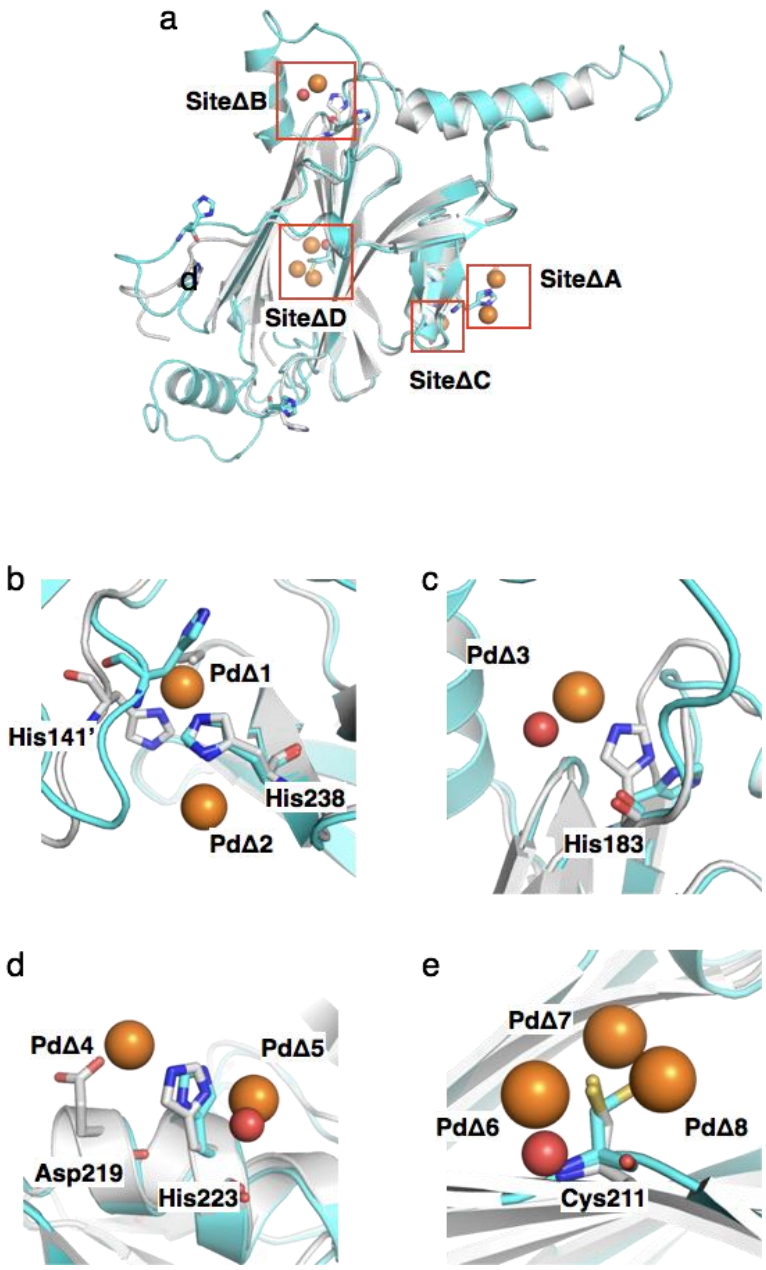

Figure S4. Superimposed structures of **Pd(allyl)•ΔL4-PhC** (white) and ΔL4-PhC (cyan). (a) Monomer structures and metal binding structures of (b) Site ΔA, (c) Site ΔB, (d) Site ΔC and (e) Site ΔD. The Pd atoms are indicated by orange spherical models.

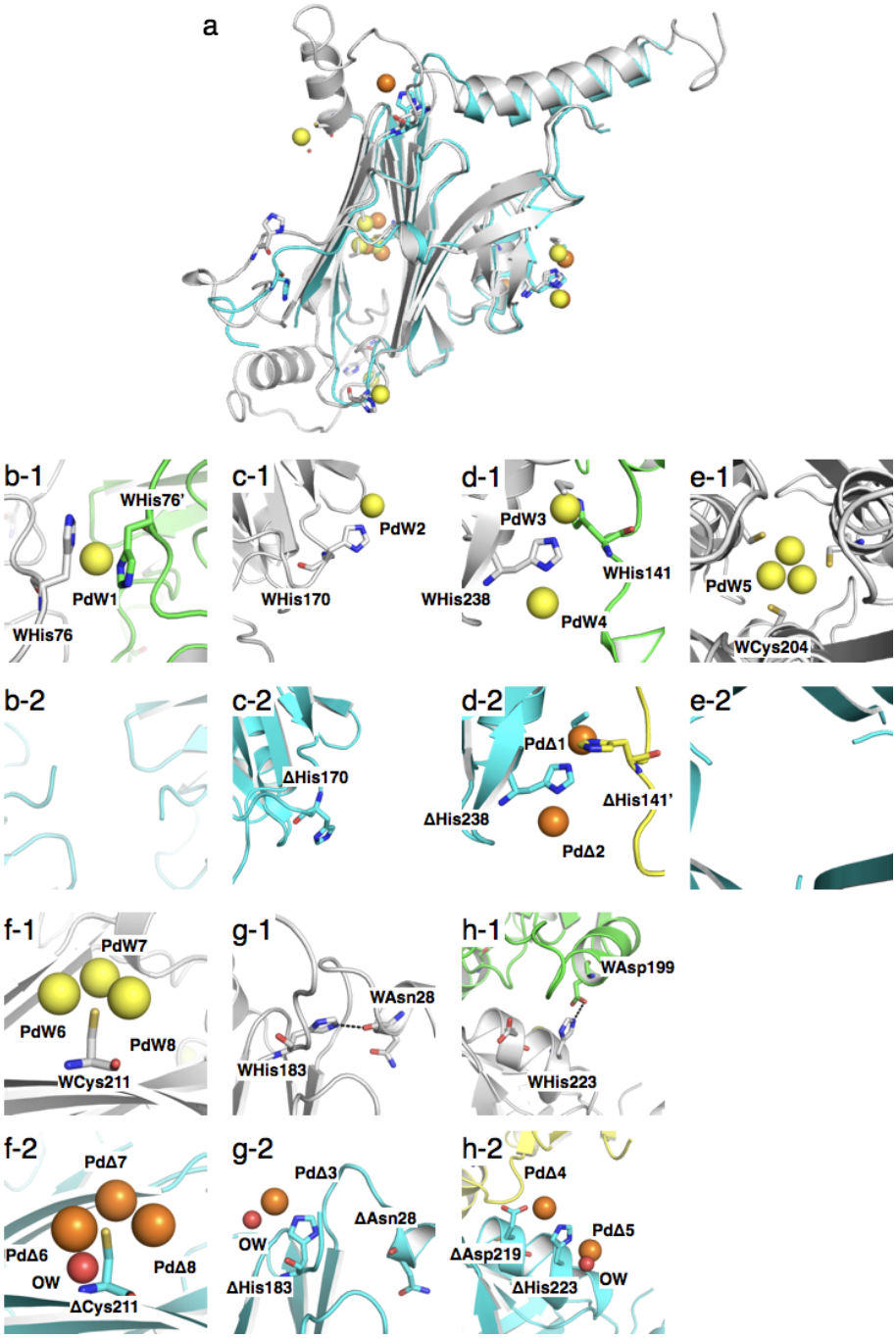

Figure S5. (a) Superimposed structures of **Pd(allyl)•WTPhC** (white) and **Pd(allyl)•ΔL4-PhC** (cyan). Pd atoms in **Pd(allyl)•WTPhC** and **Pd(allyl)•ΔL4-PhC** are indicated as yellow and orange spherical models, respectively. (b-h) Detail structures of metal binding sites. The structures of **Pd(allyl)•WTPhC** and **Pd(allyl)•ΔL4-PhC** are indicated as b1-h1 and b2-h2, respectively. The neighboring molecules are indicated in green and yellow cartoon colors in **Pd(allyl)•WTPhC** and **Pd(allyl)•ΔL4-PhC**, respectively.

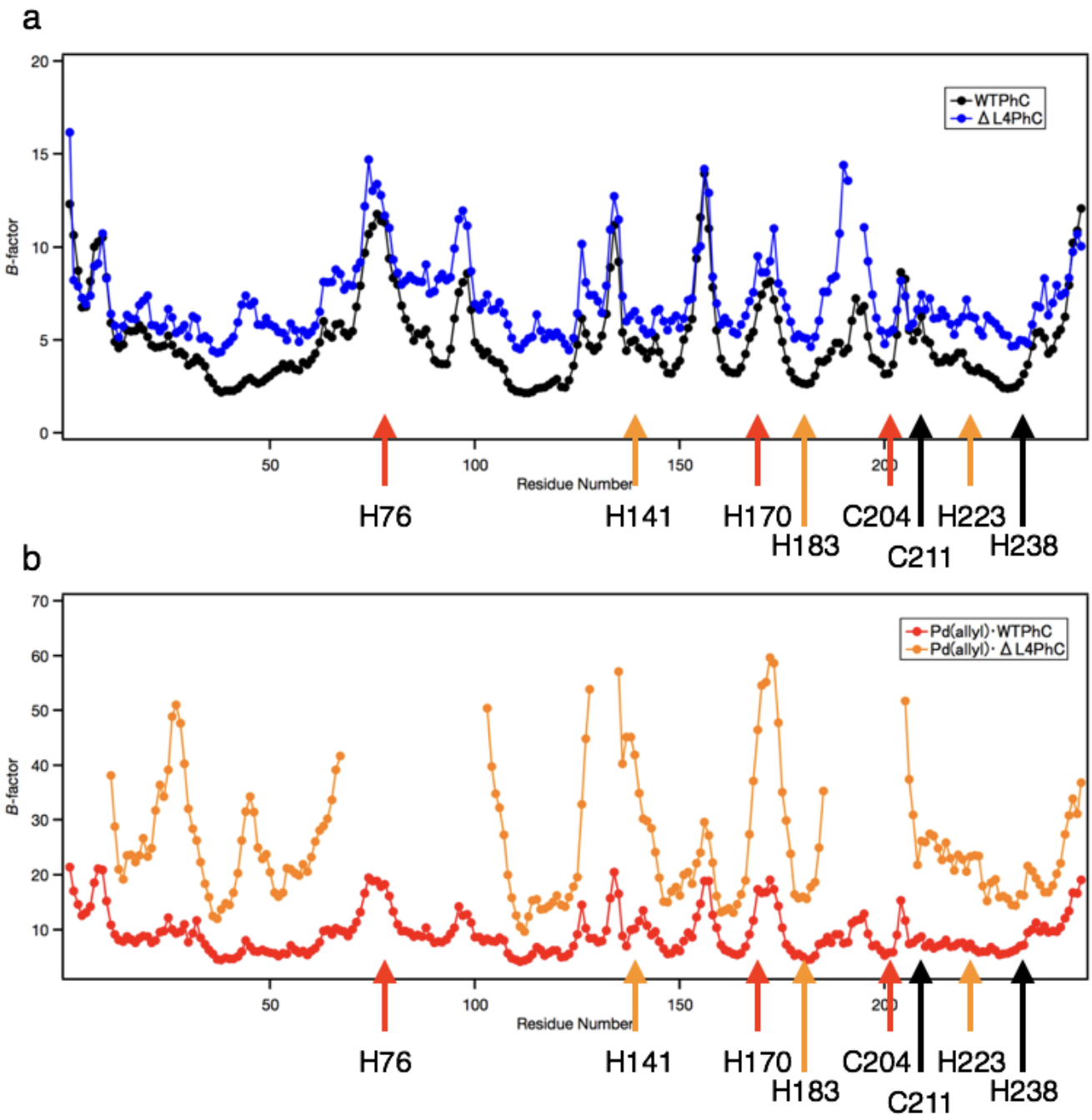

Figure S6. Average B-factors for main chain atoms of (a) WTPhC and ΔL4-PhC, (b) **Pd(allyl)•WTPhC** and **Pd(allyl)•ΔL4-PhC.** Arrows represent metal binding sites.

Table S1. Crystallographic data

Data collection	**Pd(allyl)•WTPhC**	**Pd(allyl)•ΔL4-PhC**
No. collected datasets	303	296
No. of merged datasets	97	129
Space group	*I23*	*I23*
Crystal cell (Å)		
$a = b = c$	103.2	105.9
Resolution range (Å)	50-1.58 (1.68-1.58)	50-2.08 (2.21-2.08)
Completeness (%)	100 (100)	100 (100)
Multiplicity	46.2 (46.0)	68.9 (69.1)
Unique reflections	48640 (8155)	23047 (3781)
R_{meas}	0.763 (3.58)	0.908 (14.8)
I/σ	7.31 (1.11)	9.78 (0.95)
CC1/2	0.990 (0.355)	0.994 (0.386)

Values in parentheses are for the highest-resolution shell. Friedel pairs are treated as different reflections.

Table S2. Refinement statistics.

	Pd(allyl)•WTPhC	**Pd(allyl)•ΔL4-PhC**
Resolution range (Å)	42.1-1.58	43.2-2.08
Reflection used	22665	10839
R-factor (%)	15.0	18.8
Free *R*-factor (%)	19.7	24.3
R.m.s. deviations from ideal		
Bond length (Å)	0.020	0.015
Angle (°)	1.912	1.766
Ramachandran plot (%)		
most favored	97.1	97.6
allowed	2.9	2.4

References

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