Supporting information

Polymerization of Phenylacetylene by Rhodium Complexes within a Discrete Space of apo-Ferritin

Satoshi Abe, Kunio Hirata, Takafumi Ueno*, Kazuhide Morino, Nobutaka Shimizu, Masaki Yamamoto, Masaki Takata, Eiji Yashima, and Yoshihito Watanabe*

Experiment Section

Materials. Reagents were purchased from TCI, Wako, Nacalai Tesque, and Sigma-Aldrich and used without further purification except for phenylacetylene. (4-carboxyphenyl)acetylene and ethyl(4-ethnylphenyl)phosphonate were prepared according to the previously reported method.[1] 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 literature.[2]

Preparation of Rh(nbd)•apo-Fr. An acetonitrile solution of $[Rh(nbd)Cl]_2$ (1 mL, 10 mM) was added to an apo-Fr buffer solution (10 mL, 10 μM in 50 mM Tris/HCl pH 8.0, 0.15M NaCl) and stirred for 1h at 25 °C. After dialysis against a 0.15 M NaCl aqueous solution, the mixture was purified by gel filtration (Superdex G-200) equilibrated in a 0.15M NaCl aqueous solution. The number of Rh atoms in apo-Fr was determined by ICP-OES and bicinchoninate (BCA) methods. In order to prove the reproducibility of the composite synthesis, three independent preparations were performed for the composite.

Crystallization of Rh(nbd)•apo-Fr. Crystallization of **Rh(nbd)•apo-Fr** was performed with a hanging drop vapor diffusion method as described in perevious reports.[3-5] A solution of **Rh(nbd)•apo-Fr** was concentrated to approximately 20 mg ml^{-1} and the drops were prepared by mixing on equal volume (3 μL) of a protein solution (20 mM Tris/HCl pH8.0, 0.15 M NaCl) and the precipitant solution (1 M $(NH_4)_2SO_4$, 20 mM $CdSO_4$), and equilibrated against the precipitant solution (1 mL) at

20℃. The crystals were obtained within a day.

X-ray Crystal Analysis. Before the data collection of **Rh(nbd)•apo-Fr**, single crystal was immersed in a precipitant solution containing 30 % (w/w) ethylene grycol and subsequently frozen in liquid nitrogen. X-ray diffraction data were collected at 100 K at beamline BL41XU at SPring-8 using X-ray wavelengths of 0.5334 Å and 0.4639 Å which represent the peak wavelengths of Rh and Cd X-ray absorption, respectively, to distinguish Rh atoms from Cd atoms which are essential for the crystallization.[6] Data were processed with the program *HKL2000* in the cubic F432 space group. The crystal parameters and data collection statistic are summarized in Table S1.

Refinement. The structures were determined by molecular replacement with *MOLREP* using an apo-Fr structure (pdb ID: 1DAT) as the initial model. Refinement of the protein structure was performed using *REFMAC5*[7] in the *CCP4* suite. Rebuilding was performed using *COOT*[8] based on sigma weighted ($2F_o$-F_c) and (F_o-F_c) electron density maps. About 5% of the observed data were excluded from the refinements and used to calculate the free R (R_{free}) as a monitor of model bias. Water molecules were positioned to fit residual (F_o-F_c) density peaks with a lower cut-off of 3σ. The models were subjected to quality analysis during the various refinement stages with omit maps and *PROCHECK*.[9] The refinement statistics are summarized in Table S1. The Cd binding positions are almost identical to those of the previously reported structures of apo-Fr.[4]

Polymerization using Rh(nbd)•apo-Fr. Polymerization reactions of phenylacetylene catalyzed by **Rh(nbd)•apo-Fr** were investigated at 25 °C for 3h in glass ampoules under an argon atmosphere. The reactions were performed in a 0.15 M NaCl aqueous solution containing phenylacetylene (1.5 mM), NaOH (0.3 mM), and **Rh(nbd)•apo-Fr** (0.5 μM). After three hours, the identification of products was carried out by UV-vis and **Rh(nbd)•apo-Fr** including polyphenylacetylene are purified by G200. The resulting polymers were extracted with chloroform for SEC and NMR analyses after dissociation of the composites into subunits at pH 2.0. The molecular weight of polyphenylacetylene was estimated by SEC with polystyrene standards using chloroform as the eluent. In order to determine the errors of SEC and UV data, each polymerization reaction was carried out three times.

Time Course for Polymerization. The time course for the polymerization reaction of phenylacetylene was determined in a 0.15 M NaCl aqueous solution containing phenylacetylene (3.0 mM), NaOH (0.3 mM), and **Rh(nbd)•apo-Fr** (0.5 μM) under an argon atmosphere. The molecular weight and UV-vis spectra were measured after 3, 6, 9, and 12 hours. The dependences of absorbance at 383nm of reaction mixtures on the polymerization time are given in Figure S4. The final polymer yield was calculated to be 14 % of the starting monomers after 18 hours. In order to confirm the reproducibility, the polymerization reaction was carried out three times.

Physical Measurements. Absorption spectra were recorded on a Shimazu UV-2400PC UV-vis spectrometer. Metal concentrations of **Rh(nbd)•apo-Fr** and Rh^{III}•apo-Fr were determined by using an inductively coupled plasma optical emission spectrometer (Varian ICP-OES Vista-PRO). Rh in 4.9 wt% HCl (1.011 g/L) was used as calibration standard and $Y(NO_3)_3$ in 0.1 M HNO_3 (1.01 g/L) was applied for an internal standard. Size exclusion chromatography (SEC) was performed at 40 °C with a Jasco PU-980 liquid chromatograph equipped with a UV-vis (383 nm, Jasco UV-970) detector. A Tosoh TSKgel MultiporeH_{XL}-M SEC column (30 cm) was connected and $CHCl_3$ was used as the eluent at a flow rate of 1.0 mL/min. The molecular weight calibration curve was obtained with standard polystyrenes. ^{1}H-NMR spectra were recorded on a JEOL JNM-A 600 NMR.

Table S1. Summary of X-ray Data and Refinement Statistics for **Rh(nbd)•apo-Fr**

	Rh(nbd)•apo-Fr	
	Rh Peak	**Cd Peak**
Data collection statistics		
X-ray source	BL41XU	BL41XU
X-ray wavelength, Å	0.5334	0.4639
Space group	*F*432	*F*432
Unit cell, Å	181.27	181.42
Resolution range (outer shell) (Å)	50-1.80 1.86-1.80	50.0-1.90 1.97-1.90
Total observations	519,616	441,670
Unique reflections	44,669	38,009
Completeness (%)[a]	100 (100)	100(100)
R_{merge} (%)[a,b]	7.3 (32.7)	7.6 (31.7)
$I/\sigma(I)$ [a]	38.2 (9.3)	37.9 (10.4)
Refinement statistics		
Resolution (Å)	37.0-1.80	
No. reflections Working set/test set	22,956/1,250	
R-facror (%)[c]	17.5	
R_{free} (%)[d]	19.6	
Final model		
No. of residues	1-173	
No. of water molecules	282	
No. of Sulfate ions	2	
No. of Rh atoms	3	
No. of Cd atoms	5	
No. of ethylene glycol	8	
rms diviation from ideality		
Bonds (Å)	0.011	
Angle (°)	1.140	
Ramachandran plot (%)[e]		
most favored	93.5	
allowed	6.5	

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

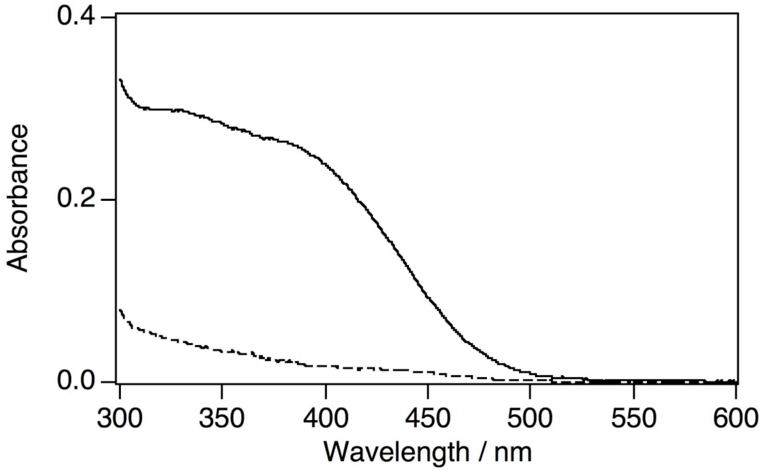

Figure S1. UV-vis spectra of **Rh(nbd)•apo-Fr** (dot line) and **Rh(nbd)•apo-Fr** after the reaction with phenylacetylene (solid line): 0.5 μM of **Rh(nbd)•apo-Fr** in 0.15 M NaCl and **Rh(nbd)•apo-Fr** treated with phenylacetylene (1.5 mM) for 3h at 25 °C in 0.15 M NaCl.

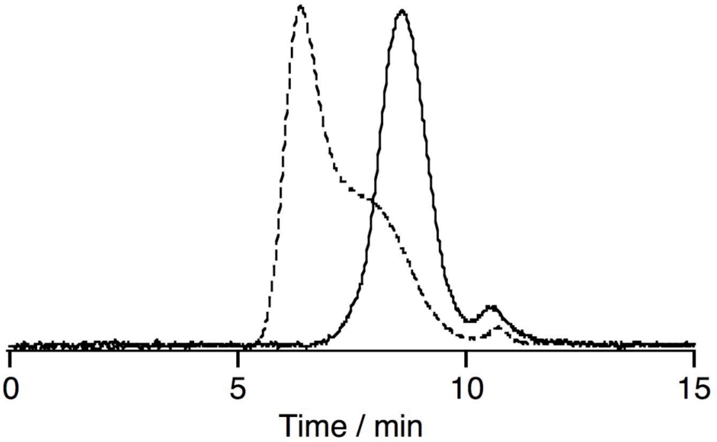

Figure S2. Elution profiles of size exclusion chromatography of polyphenylacetylene catalyzed by **Rh(nbd)•apo-Fr** (solid line) and by [Rh(nbd)Cl]$_2$ (dot line) in aqueous solution. Molecular weights are obtained based on the calibration of standard polystyrenes. Elution was monitored at 383 nm.

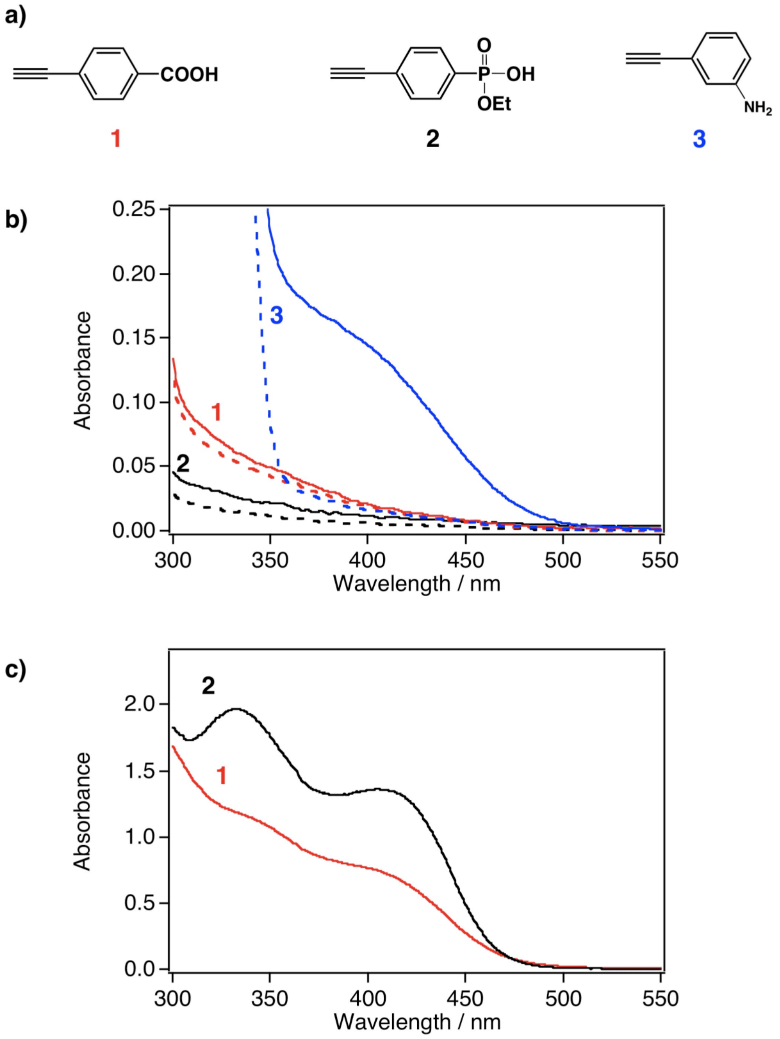

Figure S3. (a) Structures of 4-carboxyphenylacetylene (**1**), ethyl(4-ethynylphenyl) phosphonate (**2**), and 3-aminophenylacetylene (**3**). (b) UV-vis spectra before (dashed line) and after (solid line) the polymerization of **1**, **2**, and **3** catalyzed by **Rh(nbd)•apo-Fr** are shown in red, black, and blue lines, respectively. (c) UV-vis spectra after the polymerization of **1** and **2** catalyzed by [Rh(nbd)Cl]$_2$ are shown in red and black line, respectively.

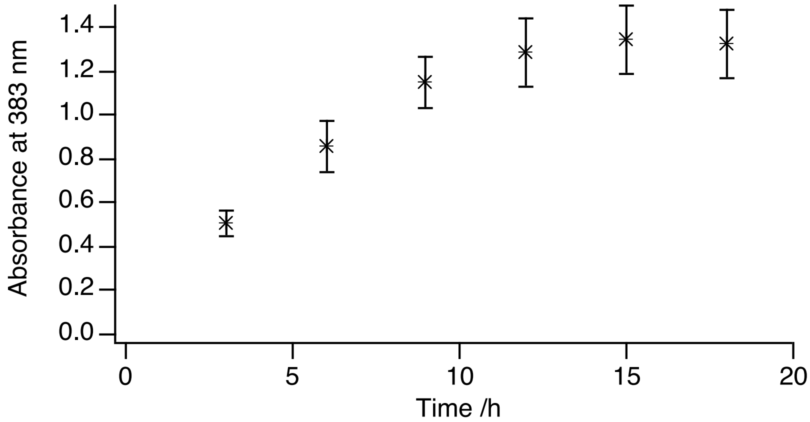

Figure S4. Time course for polymerization of phenylacetylene by **Rh(nbd)•apo-Fr**. Conditions: [**Rh(nbd)•apo-Fr**] = 0.5 μM, [phenylacetylene] = 3.0 mM, [NaOH] = 0.3 mM at 25 °C.

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