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Supplementary Information

Regulation of O_2 Evolution Reaction using Composites of Liposome and Lipophilic Ruthenium Complexes

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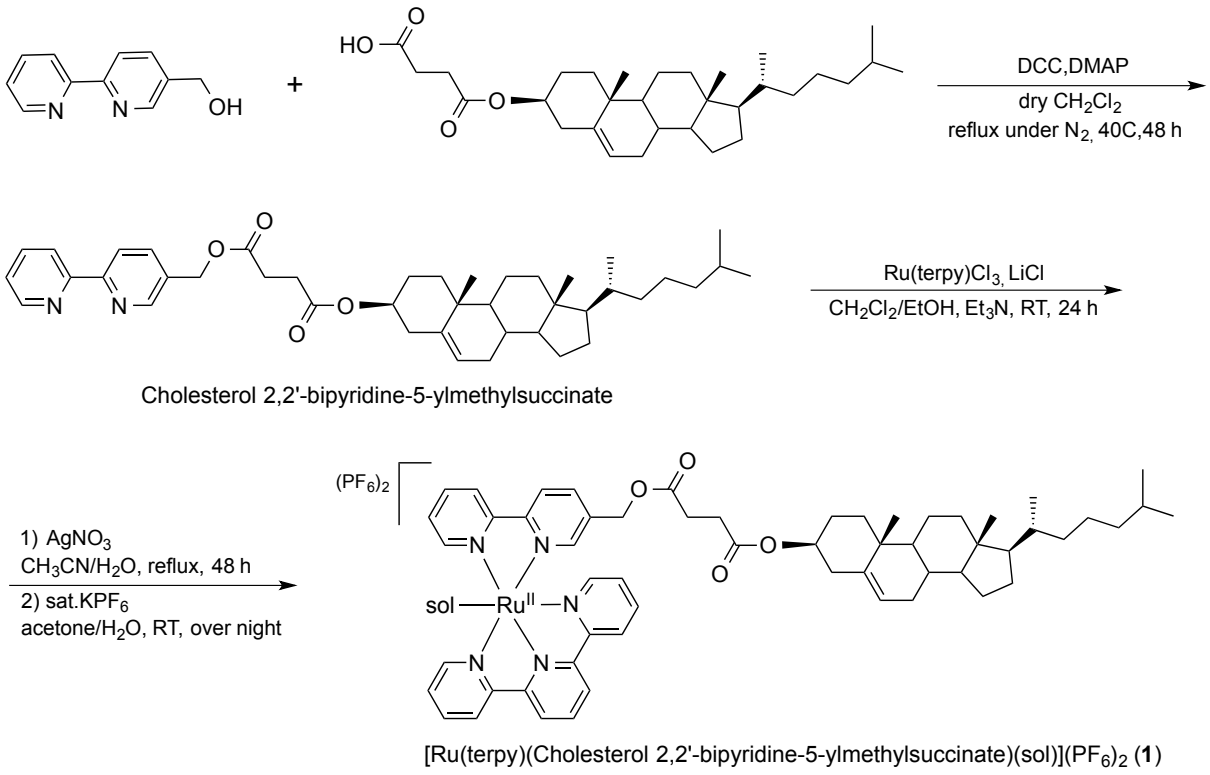
Materials. Reagents were purchased from Wako, TCI, Nacalai Tesque and Sigma-Aldrich and used without further purification. Phospholipids were obtained from Avanti Polar Lipids.

Synthesis of [Ru(terpy)(Cholesterol 2,2'-bipyridine-5-ylmethylsuccinate)(sol)]$(PF_6)_2$ (1)



Scheme S1. The synthesis of **1**.

Ethyl 2,2'-Bipyridinyl-5-carboxylate,[1] 2,2'-Bipyridinyl-5-methanol,[2] *O*-succinyl cholesterol,[3] and $Ru(terpy)Cl_3$[4] were prepared by literature methods.

Cholesterol 2,2'-bipyridine-5-ylmethylsuccinate

The solution of 2,2'-Bipyridinyl-5-methanol (100 mg) in dry dichloromethane (10 ml) was added to *O*-succinyl cholesterol (275 mg) and *N,N'*-Dicyclohexylcarbodiimide (305 mg). After *N*,*N*-dimethyl-4-aminopyridine (6.0 mg) was added to the mixed solution, the reaction mixture was stirred at 40 ˚C for 48 h and then cooled to 0 ˚C in ice bath. The solution was filtered with celite for the removal of insoluble materials. Then the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (1:50 methanol/ dichloromethane). The resulting brown oil contained cholesterol 2,2'-bipyridine-5-ylmethylsuccinate and unreacted *O*-succinyl cholesterol as impurity, which

was used in the next reaction without further purification.

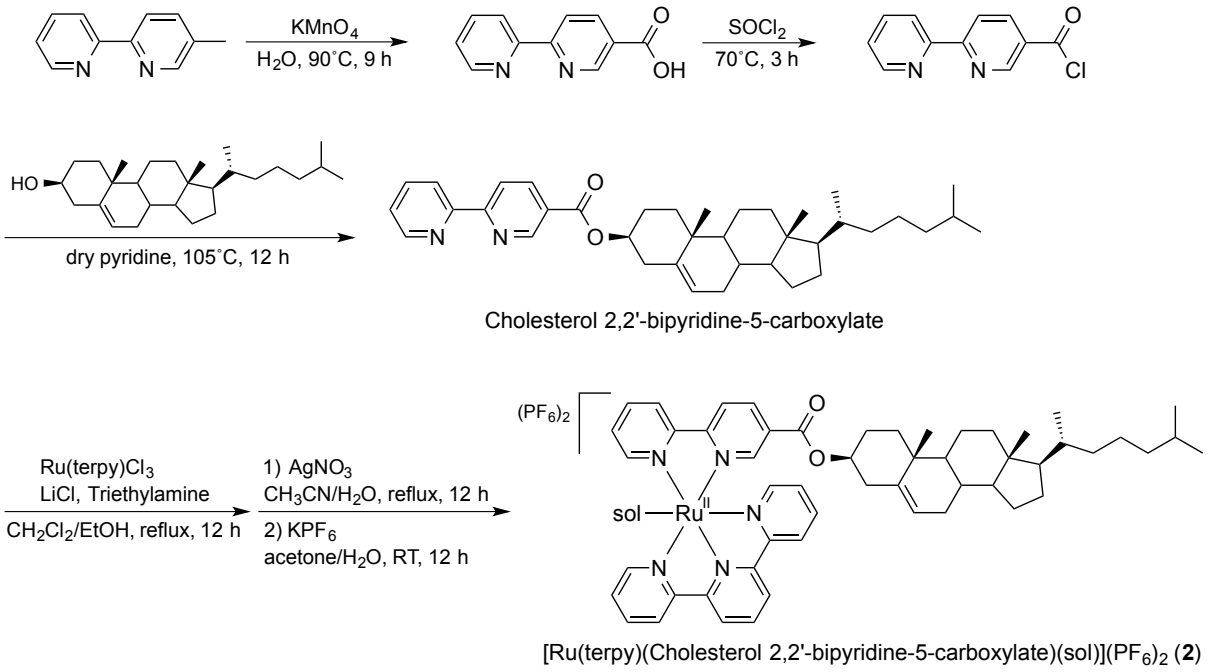
Yield 88 %. ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.66 (s, 3H, -CH_3), 0.85 – 1.68 (m, 44H, -CH-, -CH_2-, -CH_3), 1.79 – 2.00 (m, 7H, -CH-, -CH_2-), 2.26 (m, 2H,), 2.63 (m, 2H,), 2.70 (m, 2H,), 4.58 -4.60 (m, 1H, -O-CH-), 5.21 (s, 2H, -CH_2-), 7.34 (s, 1H), 7.83-7.84 (m, 2H), 8.43 (m, 2H), 8.69 (m, 2H) ppm.

[Ru(terpy)(Cholesterol 2,2'-bipyridine-5-ylmethylsuccinate)(sol)]$(PF_6)_2$ (1)

$Ru(tpy)Cl_3$ and LiCl were added to the solution of Cholesterol 2,2'-bipyridine-5-ylmethylsuccinate (200 mg) in dry dichloromethane (12 ml). To the mixture was added ethanol (24 ml) and trimethylamine (1.2 mL) and the mixture was stirred at RT for 48 h. The solution was filtered with celite for the removal of insoluble materials. The filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (LH-20 column, 100% dichloromethane) to obtain pure [Ru(tpy)(Cholesterol 2,2'-bipyridine-5-ylmethylsuccinate)(Cl)]Cl as a purple solid. $AgNO_3$ (60 mg, 0.35 mmol) was added in 2 portions at 24 h intervals to a solution of [Ru(tpy)(Cholesterol 2,2'-bipyridine-5-ylmethylsuccinate)(Cl)]Cl (50 mg, 0.29 mmol) in a 3:1 (v/v) acetonitrile/water mixture (64 ml). The mixture was heated at 85 ˚C in the dark for 48 h followed by filtration for the removal of AgCl. The filtrate was concentrated under reduced pressure. The residual solid was dissolved in acetone (15 mL) and an aqueous saturated KPF_6 solution was added to the filtrate. One day later, the suspension was filtered to obtain **1** as a orange-colored solid.

Yield 62.4 %. MALDI-TOF-MS: Found m/z = 1142.2 {([Ru(terpy)(Cholesterol 2,2'-bipyridine-5-ylmethylsuccinate)]$^{2+}$)+(DHB)-(H)}. calcd exact mass for $C_{64}H_{74}N_5O_8Ru$: 1142.3

Synthesis of [Ru(terpy)(Cholesterol 2,2'-bipyridine-5-carboxylate)(sol)]$(PF_6)_2$ (2)



Scheme S2. The synthesis of **2**.

2,2'-bipyridine-5-carboxylic acid [1] and 2,2'-bipyridine-5-carboxylic acid chloride [5] were prepared by literature methods.

Cholesterol 2,2'-bipyridine-5-carboxylate

The solution of cholesterol (0.19 g) in dry pyridine (2.2 ml) was added to 2,2'-bipyridine-5-carboxylic acid chloride under a nitrogen atmosphere. The reaction mixture was stirred at 110 ˚C for 9 h and then cooled to RT. The mixture was filtered for the removal of insoluble materials. The crude product was purified by column chromatography (1:2 hexane/diethyl ether). The resulting while solid contained cholesterol 2,2'-bipyridine-5-carboxylate and unreacted cholesterol as impurity, which was used in the next reaction without further purification.

Yield 10.5 %. ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.80 (s, 3H, -CH_3), 0.85 – 1.60 (m, 57H, -CH-, -CH_2-, -CH_3), 1.68 – 2.04 (m, 8H, -CH-, -CH_2-), 2.50 (dd, *J* = 12 Hz, 2H,), 4.89 – 4.94 (m, 1H), 5.40 (dd, *J* = 4.8 , 1H), 7.37 (t, *J* = 8.4,9.0 Hz, 1H), 7.53 (m, 1H), 7.70 (m, 1H), 7.86 (t, *J* = 7.8, 7.2 Hz, 1H), 8.40 (d, *J* = 2.4, 1H), 8.50 (t, *J* = 8.4, 9.0 Hz, 2H), 8.72 (dd, *J* = 4.2 Hz, 1H), 9.27 (s, 1H) ppm.

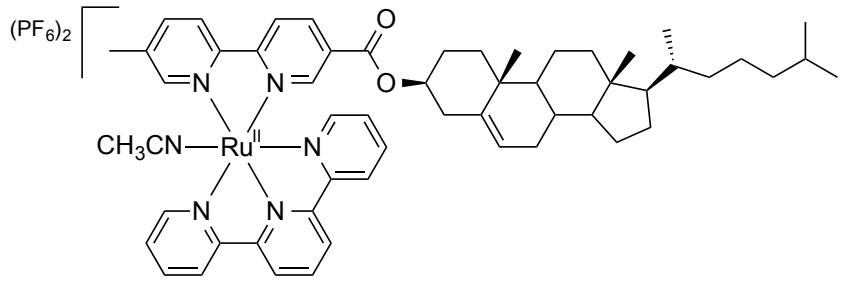
[Ru(terpy)(Cholesterol 2,2'-bipyridine-5-carboxylate)(sol)]$(PF_6)_2$ (2)

Cholesterol 2,2'-bipyridine-5-carboxylate (16.8 mg), Ru(terpy)Cl_3 (16.4 mg) and LiCl

(6.0 mg) were dissolved in 1:1 (v/v) CH_2Cl_2/ethanol mixture. To the mixture was added triethylamine (0.059 ml) as a reductant and the mixture was refluxed at room temperature for 24 h. The crude product was purified by gel chromatography (Sephadex LH-20, eluted with 100% CH_2Cl_2) to obtain pure [Ru(terpy)(Cholesterol 2,2'-bipyridine-5-carboxylate)(Cl)]Cl as a purple solid. [Ru(terpy)(Cholesterol 2,2'-bipyridine-5-carboxylate)(Cl)]Cl (14.1 mg) and $AgNO_3$ (8.4 mg) in a 3:1 (v/v) CH_3CN/H_2O mixture was stirred at 85 ˚C in the dark for 12 h followed by filtration for the removal of AgCl. Then the filtrate was concentrated under reduced pressure followed by addition of acetone. To the solution was added an aqueous saturated KPF_6 solution. The mixture was stirred at RT for 12 h. The orange precipitate was collected by filtration, washed with cold water, and dried in vacuo.

Yield 21.7 %. MALDI-TOF-MS: Found m/z = 922.1 {[Ru(terpy)(Cholesterol 2,2'-bipyridine-5-carboxylate)(H_2O)]$^{2+}$}, calcd exact mass for $C_{53}H_{65}N_5O_3Ru$: 921.2.

Synthesis and crystallization of [Ru(terpy)(Cholesterol 5'-methyl-2,2'-bipyridine-5-carboxylate)(CH_3CN)](PF_6)$_2$



[Ru(terpy)(Cholesterol 5'-methyl-2,2'-bipyridine-5-carboxylate)(CH_3CN)](PF_6)$_2$

5'-methy-2,2'-bipyridine-5-carboxylic acid,[6] 5'-methy-2,2'-bipyridine-5-carboxylic acid chloride[5] were prepared by literature methods. The synthesis of [Ru(terpy)(Cholesterol 5'-methyl-2,2'-bipyridine-5-carboxylate)(CH_3CN)](PF_6)$_2$ utilized the same procedure as that of **2**. Orange single crystals obtained from acetone/water mixture.

Yield 31.8 %. MALDI-TOF-MS: Found m/z = 1070.6 {([Ru(terpy)(Cholesterol 5'-methyl-2,2'-bipyridine-5-carboxylate)]$^{2+}$)+(DHB)-(H)}. Calcd. exact mass for $C_{54}H_{67}N_5O_3Ru$: 1070.2

Preparation of Liposome Composites.

Liposome composites were prepared from lipid chloroform/methanol solution containing phospholipid with a stearoyl group (87.4 mol%), DSPE-PEG_{2000} (3.6 mol%) and lipophilic Ru

complex (9 mol%). The organic solvent was removed by rotary evaporation yielding a thin lipid film on the sides of a round bottom flask. They were placed under vacuum overnight to remove residual organic solvent. The lipid film was hydrated with 1.0 mL of milliQ water at 65 ˚C (DSPC, DSPG) or 85 ˚C (DSPA). The final concentration of the lipid was 5.0 mM. The lipid suspensions were sonicated for 40 minutes and then extruded 10 times at 60 ˚C (DSPC, DSPG) or 80 ˚C (DSPA) through 100 nm polycarbonate filters. The ruthenium concentrations of composites were determined by ICP-MS (Agilent 7500c).

O_2 Evolution Studies. O_2 evolution was carried out as described previously.[7] The reaction was initiated by adding a solution of a liposome composite (0.32 mM) in water (500 μL) to a solution of $Ce(NH_4)_2(NO_3)_6$ (0.26 M) in water (1500 μL) at 20 °C under Ar atmosphere. The amount of O_2 evolved was monitored using an YSI model 5300 oxygen meter. Prior to the measurements, a calibration of the oxygen meter was repeatedly carried by injecting a known amount of pure O_2 gas into the measurement cell. An H-shape system consisting of two flat bottom glass tubes, which has an inner volume of about 15 mL, was employed in the measurements. One compartment was equipped with an oxygen meter and the other was sealed with a rubber septum, which was used to introduce a catalyst solution using the syringe technique. An oxygen meter was immersed in water (3 mL). The two solutions inside the H tube were associated with each other only through the gas above the solutions to have the same O_2 concentration during the measurements. The solutions were both immersed in a water bath thermostated at 20 ºC.

Physical Measurements. UV-visible absorption spectra were recorded on a JASCO V-630 Bio spectrophotometer. ^{1}H NMR spectra were recorded on a JEOL ECA-600 spectrometer. MALDI-TOF mass spectra were measured on a Bruker Autoflex. 2, 5-dihydroxybenzoic acid was used as a matrix. The size distribution and Zeta potential of the liposomes was measured by Dynamic light scattering (DLS) Zetasizer Nano ZS (Malvern Instruments Ltd). The $Z_{average}$ diameter values are the means of 3 repeat measurements. The zeta potential values are the means of 4 repeat measurements. Cyclic voltammograms were recorded on a BAS CV-50W cyclic voltammetric analyzer. Samples were dissolved in $CHCl_3$, then were deposited on a glassy carbon working electrode (diameter = 3 mm). The electrodes were immersed in 0.5 M H_2SO_4 solution under N_2 atmosphere, and cyclic voltammograms were recorded at a scan rate of 10 mV/s (counter electrode: Pt wire, reference electrode: Ag/AgCl). Confocal measurements were performed using a Nikon C2si confocal microscope.

Crystal Structure Analysis of [Ru(terpy)(Cholesterol 5'-methyl-2,2'-bipyridine-5-carboxylate)(CH_3CN)](PF_6)$_2$.

Single-crystal X-ray data were recorded on a Bruker SMART APEX II ULTRA CCD Diffractometer with confocal monochromated Mo-Kα radiation. The structure was solved by a direct method and refined by full-matrix leastsquares refinement using the SHELXL-2014/6 computer program. There are some disorder in the structure. The disordered atoms except for Ru and P were refined as isotopically and the other non-hydrogen atoms were refined anisotropically. The hydrogen atoms were refined geometrically by using a riding model. (CCDC 1050758)

Table S1 Crystal parameters

Temperature	100 K
Formula	$C_{56}H_{68}F_{12}N_6O_2P_2Ru$
F. W.	1248.18
Crystal system	Triclinic
Space group	*P*1
a / Å	10.613(4)
b / Å	12.785(5)
c / Å	23.303(9)
α / °	102.430(5)
β / °	97.226(5)
γ / °	106.143(5)
V / Å^3	2907.4(19)
Z	2
D / g cm^{-3}	1.426
R	0.0742
R_w	0.1985

References

1 N. C. Fletcher, M. Nieuwenhuyzen and S. Rainey, *J. Chem. Soc., Dalton Trans.,* 2001, 2641-2648.

2 S. G. Telfer, G. Bernardinelli and A. F. Williams, *Dalton Trans.*, 2003, 435-440.

3 P. Pescador, N. Brodersen, H. A. Scheidt, M. Loew, G. Holland, N. Bannert, J. Liebscher, A. Herrmann, D. Huster, and A. Arbuzova, *Chem. Commun.*, 2010, **46,** 5358-5360.

4 Y. Halpin, D. Dini, H. M. Y. Ahmed, L. Cassidy, W. R. Browne, and J. G. Vos, *Inorg. Chem*., 2010, **49**, 22799-2807.

5 Y. Katoh, Y. Tsujimoto, C. Yamamoto, T. Ikai, M. Kamigaito, and Y. Okamoto, *Polymer Journal*, 2011, **43**, 84-90.

6 C. B. Spillane, N. C. Fletcher, S. M. Rountree, H. Berg, S. Chanduloy, J. L. Morgan, and F. R. Keene, *J. Biol. Inorg. Chem.*, 2007, **12**,797-807.

7 S. Masaoka and K. Sakai, *Chem. Lett.*, 2009, **38**, 2.

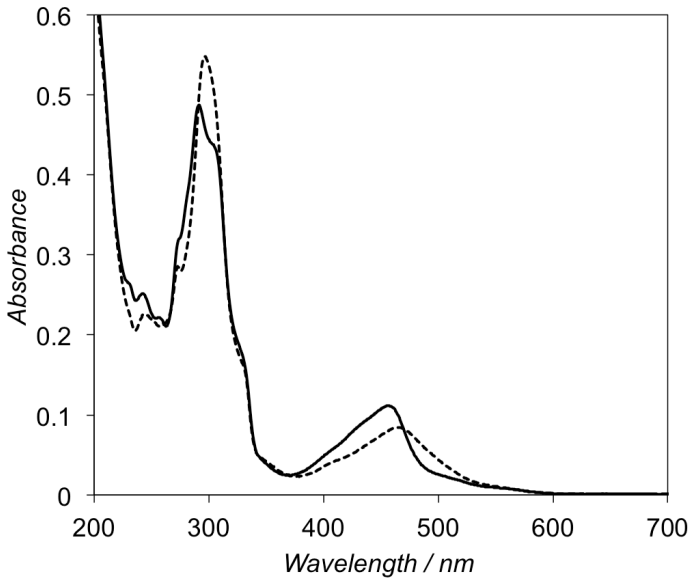


Fig. S1 UV-visible spectra of **1** (solid line) and **2** (dashed line) in acetonitrile (10 μM).

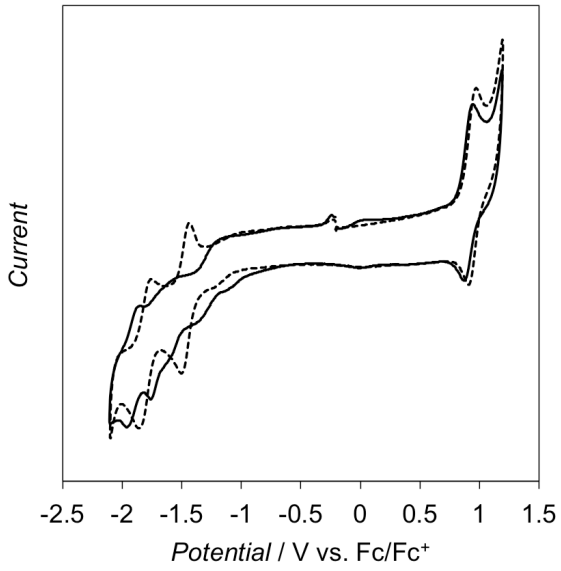


Fig. S2 Cyclic voltammograms of **1** (solid line, 0.5 mM) and **2** (dashed line, 0.5 mM) in acetonitrile under N_2 atmosphere, recorded at a scan rate of 10 mV/s using a glassy carbon working electrode, a Pt wire counter electrode, and a [Ag]/[AgCl] reference electrode. The supporting electrolyte was 0.1 M tetra(n-butyl)ammonium perchlorate. The potentials were corrected by ferrocene/ferrocinium couple as an internal standard.

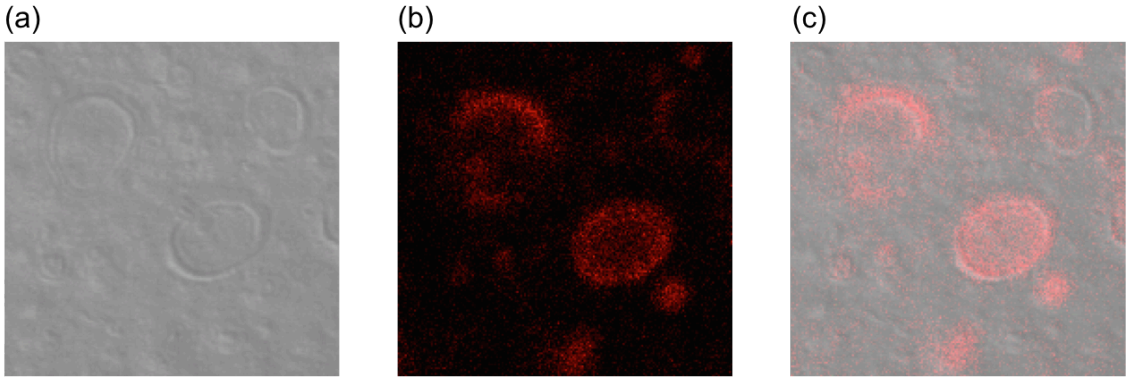


Fig. S3 Confocal laser scanning microscopy images of **1**•PA, (a) bright field, (b) fluorescence, and (c) merged. Giant vesicles of **1**•PA were prepared by film hydration method. **1**•PA was excited with 405 nm, 488 nm, and 561 nm lasers. Fluorescence from **1** is shown in red.

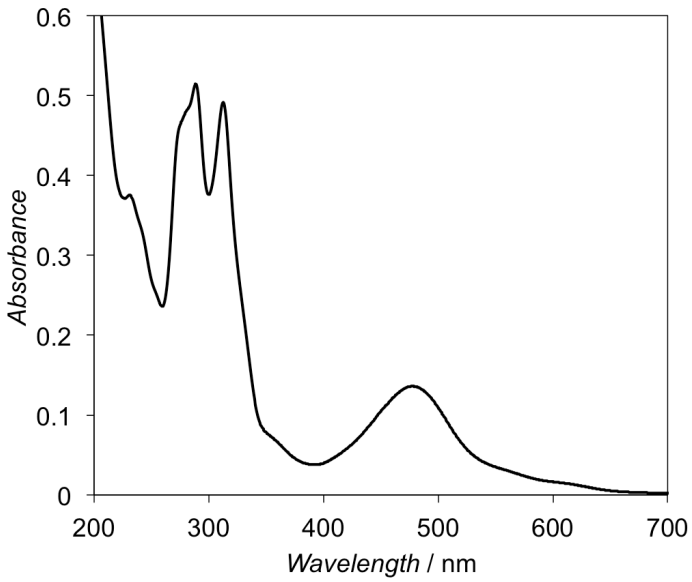


Fig. S4 UV-visible spectrum of **cRu** in 50 mM Na_2HPO_4. The sample heated at 85 ℃ for 19 h before the measurement.

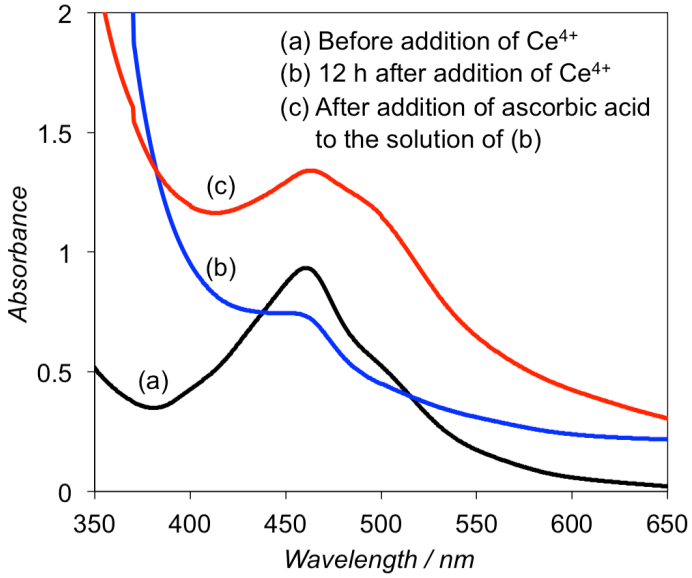


Fig. S5 UV-visible spectra of (a) **1**•PG (Ru concentration 0.08 mM) in water, (b) the reaction mixture after 12 h from addition of $(NH_4)_2[Ce(NO_3)_6]$ (3.2 mM, 40 equiv.) to **1**•PG (Ru concentration 0.08 mM), and (c) the solution after addition of ascorbic acid (1.9 mM) to the solution of (b).

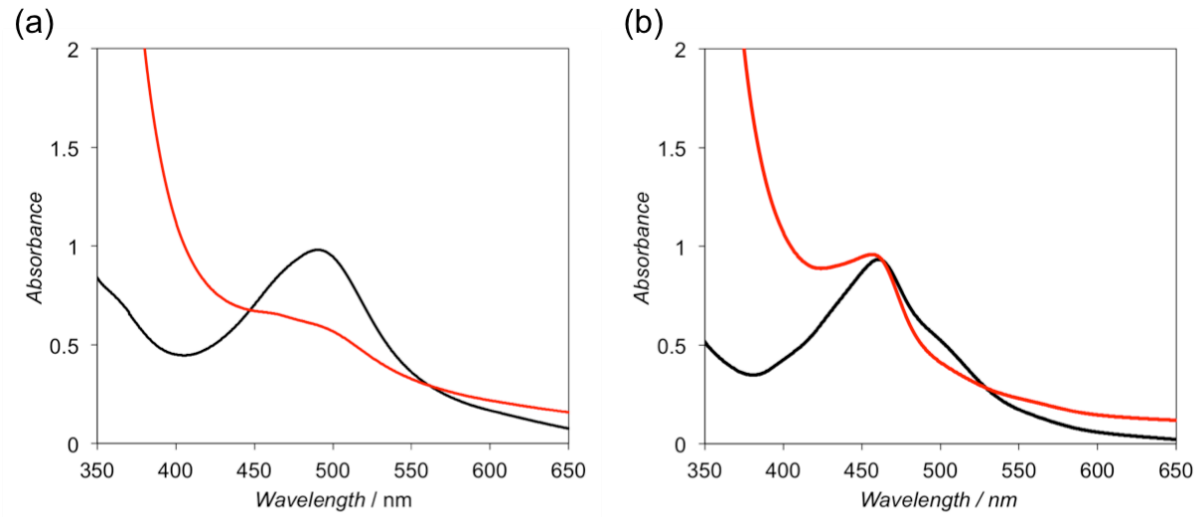


Fig. S6 UV-visible spectra of (a) **1**•PA (Ru concentration 0.08 mM) in water (black) and the mixture of **1**•PA (Ru concentration 0.08 mM) and $(NH_4)_2[Ce(NO_3)_6]$ (3.2 mM, 40 equiv.) (red), and (b) **1**•PG (Ru concentration 0.08 mM) in water (black) and the mixture of **1**•PG (Ru concentration 0.08 mM) and $(NH_4)_2[Ce(NO_3)_6]$ (3.2 mM, 40 equiv.) (red).