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Supporting Information

Selective Purification and Chemical Labeling of Target Protein on Ruthenium Photocatalyst-immobilized Affinity Beads

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1. Experimental section

General. NMR spectra were recorded on a Bruker biospin AVANCE III (500 MHz for ^{1}H, 125 MHz for ^{13}C) instrument in the indicated solvent. Chemical shifts are reported in units parts per million (ppm) relative to the signal (0.00 ppm) for internal tetramethylsilane for solutions in $CDCl_3$ (7.26 ppm for ^{1}H, 77.0 ppm for ^{13}C) or CD_3CN (1.94 ppm for ^{1}H, 118.26 ppm for ^{13}C). Multiplicities are reported using the following abbreviations: s; singlet, d; doublet, dd; doublet of doublets, t; triplet, q; quartet, m; multiplet, br; broad, *J*; coupling constants in Hertz. IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. Only the strongest and/or structurally important peaks are reported as IR data given in cm^{-1}. High-resolution mass spectra (HRMS) were recorded on a Bruker ESI-TOF-MS (micrOTOF II). Analytical thin layer chromatography (TLC) was performed on a glass plate of silica gel 60 GF254 (Merck). Silica gel (Fuji Silysia, CHROMATOREX PSQ 60B, 50-200 μm) was used for column chromatography. Reverse phased column chromatography was performed with GL Science InterSep C18. Most commercially supplied chemicals were used without further purification. All chemicals and purified proteins for biological experiments were obtained from commercial sources and used without further purification. Compound **5** was purchased from Tamagawa Seki.

Preparation of Ru cat. and ligand immobilized FG beads.[1] 0.5 mg of FG-NHS beads (purchased from Tamagawa Seiki Co. Ltd.) was washed three times with 100 μL of DMF. To the FG-NHS beads in DMF was added Ru cat. **2 - 4** (from 10 mM solution in DMF, final concentration 0.1 mM), affinity ligand (**1**: from 10 mM solution in DMF, **5**: from 0.2 mM solution in DMF, final concentration 0.1 mM) and Et_3N (from 50 mM solution in DMF, final concentration 1 mM) (final concentration of beads: 5.0 mg/mL). After stirring at room temperature for 1 h, the supernatant was removed by centrifugation (13200 rpm, 4 °C, 7 min) to give the beads immobilized on both Ru cat. and affinity ligand. Then, to the reaction mixture was added 4-aminobutanol (final 1 M in DMF) and stirred at room temperature for 1 h to convert unreacted NHS ester to alcohol. The supernatant was removed by centrifugation (13200 rpm, 4 °C, 7 min), the beads was washed with Lysis buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 v/v% Triton X) three times and applied next step.

Determination of density of ruthenium photocatalysts and ligand. The density of **1-5** immobilized on beads was determined by absorbance measurement of supernatant collected after immobilization reaction. The wavelength of 254 nm (**1**), 340 nm (**5**) and 430 nm (**2-4**) were applied.

Transmission electron microscope imaging of the beads immobilized with 4.

The beads were observed by a transmission electron microscope (JEM-1400Plus, JEOL Ltd.) at an

acceleration voltage of 80 kV. Digital images (2048 × 2048 pixels) were taken with a CCD camera (VELETA, Olympus Soft Imaging Solutions GmbH).

Preparation of crude cellular extract. HeLa cells (2.0×10^7 cell) was washed with phosphate buffered saline three times and added to 1 mL of EDTA. After incubation at 37 °C for 5 min, the suspension was added to 4 mL of phosphate buffered saline and HeLa cells and supernatant were removed by centrifugation (1500 rpm, r.t., 3 min). HeLa cells were washed with phosphate buffered saline three times and 1.0 mL of Lysis buffer was added. After incubation on the ice for 15 min, the sample was vortexed for 10 s, centrifuged (13200 rpm, 4 °C, 7 min) to give supernatant as HeLa lysate. The concentration of HeLa lysate was determined by protein BCA assay.

CA purification from the protein mixture with beads.[1] HeLa lysate was centrifuged (13200 rpm, 4 °C, 30 min) before using to remove insoluble protein aggregates. 0.25 mg of the beads was added 100 μL of protein mixture (1 μM CA in 3.0 mg/mL HeLa lysate). After stirring at 4 °C for 4 h, supernatant was removed, the beads was washed with 100 μL of Lysis buffer three times, suspended in 40 μL of 1×SDS-PAGE sample buffer (50 mM Tris–HCl pH 6.8, 125 mM 2-mercaptoethanol, 2% sodium dodecyl sulfate (SDS), 0.025% bromophenol blue, 10% glycerol) and boiled at 95 °C for 5 min. The supernatant was collected by magnetic separation and analyzed by SDS-PAGE.

CA or DHFR capture from protein mixture and protein labeling. To the 0.25 mg of beads was added 100 μL of protein mixture (1 μM CA in 3.0 mg/mL HeLa lysate, 1 μM CA, OVA and BSA in 10 mM MES buffer (pH 7.4) or 3.0 mg/mL HeLa lysate) and rotated at 4 °C for 4 h. Then, the supernatant was removed by magnetic separation and wash with 100 μL of Lysis buffer three times. After this washing operation, 50 μL of 10 mM MES buffer was added to the beads. To the solution of beads was added biotin-TRT (from 100 mM solution in DMSO, final concentration 500 μM) and APS (from 100 mM solution in H_2O, final concentration 1 mM). The beads were distributed and the mixture was irradiated with blue light (RELYON, Twin LED light, 455 nm) on ice for 5 min. The reaction was immediately quenched with 5×SDS-PAGE sample buffer, the mixture was boiled at 95 °C for 5 min and the beads was removed by magnetic separation. The protein mixture was separated by SDS-PAGE, transferred to polyvinylidene difluoride (PVDF) membrane (GE Healthcare), blocked with Immuno Block (DS Pharma), treated with horseradish peroxidase (HRP)-conjugated streptavidin (streptavidin-HRP, Sigma-Aldrich), and a blot was treated with ImmunoStar LD (Wako Pure Chemical Industries, Ltd.).

CA labeling in protein mixture. To the 0.25 mg of beads was added 100 μL of protein mixture (1.0 μM CA in 1.0 mg/mL HeLa lysate) and rotated at 4 °C for 4 h. Then, to the solution of beads was added biotin-TRT (from 100 mM solution in DMSO, final concentration 500 μM) and APS (from

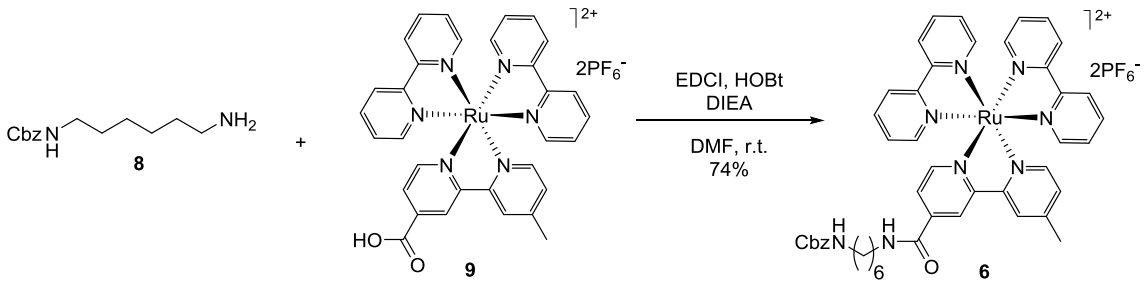
100 mM solution in H_2O, final concentration 1 mM). The solution was distributed and irradiated with blue light (RELYON, Twin LED light, 455 nm) on ice for 5 min. The reaction was immediately quenched with 5×SDS-PAGE sample buffer. Then, the mixture was boiled at 95 °C for 5 min, and analyzed by SDS-PAGE and Western-blotting according to the above-described method.

Measurement of CA activity. CA was labeled according to above-described method. After photo reaction, the reaction was immediately quenched with Tris(2-carboxyethyl)phosphine Hydrochloride (TCEP-HCl) (from 100 mM solution in DMSO, final concentration 1 mM), to the solution was added 60 mM acetate buffer (pH 5.2) containing 2 M NaCl (final concentration 30 mM acetate, 1 M NaCl) and incubation on ice for 10 min.[2] The supernatant was diluted with 10 mM MES buffer (pH 7.4) and excess amount of small molecules was removed by AmiconUltra (MWCO: 10 kDa). To the labeled CA solution (50 μL/well in 96 well plate) was added 4-nitrophenyl acetate (1.1 mM in Tris buffer 50 mM, pH 8.0, 100 μL/well in 96 well plate) and the time-dependent increase in absorbance at 340 nm (4-nitrophenol) was detected using a plate reader (TECAN, Infinite F200).

Electrochemical measurement.[3] Electrochemical measurements were made with a Hokutodenkou HZ-5000 analyzer. A three electrode system which consists of glassy carbon working electrode, a platinum wire counter electrode and an Ag/AgCl (in saturated aqueous KCl solution) reference electrode was used. The CV measurement were performed in 200 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.4) which contained 10% DMSO (for dissolution of substrate) and 1.0 mM substrate at room temperature. A scan rate of 100 mV/s was applied.

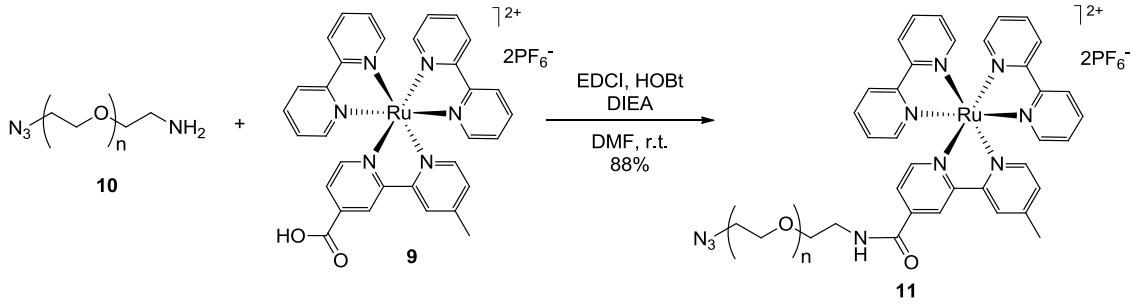
DLS measurement.[4] Particle size and zeta potential of Ru-catalyst-immobilized FG beads were measured with an electrophoretic light scattering spectrophotometer (Nano-ZS, Sysmex, Japan). Beads were suspended in H_2O and 0.5 mg/mL of beads solution was applied.

Synthesis of Compounds. *N*-(6-aminohexyl)-4-sulfamoylbenzamide (**1**), N^5-(Biotin-polyethylene glycol)-N^1-[4-(dimethylamino)phenyl]glutaramide (biotin-TRT), Bis(2,2'-bipyridine)[4'-methyl-(2,2'-bipyridine)-4-carboxylicacid]ruthenium(II) bis(hexafluorophosphate) ($Ru(bpy)_2(mcbpy)(PF_6)_2$, **9**)[5], N_3-PEG300-NH_2 (**10**)[6] and 2,2'-bipyridine-4,4'-dicarboxylic acid (dcbpy)[7] were prepared according to the previously reported procedures.



Bis(2,2'-bipyridine){Benzyl[6-(4-methyl-2,2'-bipyridyl-4'-carboxyamido)hexyl]carbamate} ruthenium(II) bis(hexafluorophosphate) (6). To a solution of **9** (63.6 mg, 0.0693 mmol), EDCI·HCl (16.1 mg, 0.0840 mmol) and HOBt (12.2 mg, 0.0903 mmol) in 3.0 mL of DMF was added **8** (24.7 mg, 0.0861 mmol) and DIEA (10.0 μL, 0.0580 mmol). After stirring at room temperature for 24 h, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in 5% MeCN/H_2O and purified with reverse-phase column chromatography (5-50% MeCN/H_2O) to give **6** as red solid (58.9 mg, 74% yield).

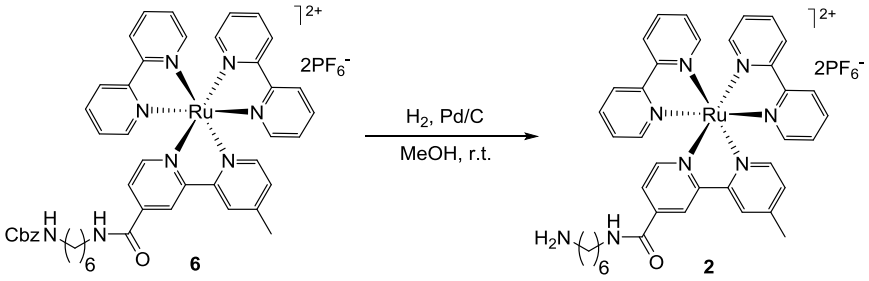
Mp >300 °C; ^{1}H NMR (500 MHz, CD_3CN) δ 8.82 (d, J = 1.5 Hz, 1H), 8.53 (s, 1H), 8.49 (d, J = 8.0 Hz, 4H), 8.07-8.04 (m, 4H), 7.83 (d, J = 6.0 Hz, 1H), 7.73-7.69 (m, 4H), 7.65 (dd, J = 1.6 Hz, 5.8 Hz, 1H), 7.55 (d, J = 6.0 Hz, 1H), 7.40-7.26 (m, 10H), 5.00 (s, 2H), 3.39-3.37 (m, 2H), 3.10-3.09 (m, 2H), 2.54 (s, 3H), 1.62-1.54 (m, 2H), 1.49-1.45 (m, 2H), 1.37-1.33 (m, 5H); ^{13}C NMR (125 MHz, CD_3CN) δ 164.0, 158.8, 157.9, 157.8, 157.7, 157.4, 156.9, 153.2, 152.6, 152.5, 151.7, 151.6, 143.6, 138.8, 138.4, 129.6, 129.3, 128.7, 128.5, 126.4, 125.5, 125.2, 122.4, 66.6, 41.2, 41.1, 30.4, 29.7, 26.9, 21.2; FT-IR (KBr) 3423, 3338, 3112, 3082, 2936, 2862, 1773, 1675, 1604, 1543, 1466, 1202, 1136 cm^{-1}; HRMS (ESI, positive): *m/z* calced. for $C_{46}H_{46}N_8O_3Ru$ $[M]^{2+}$: 430.1369, found 430.1375. See Figure S14 for UV-Vis absorbance.



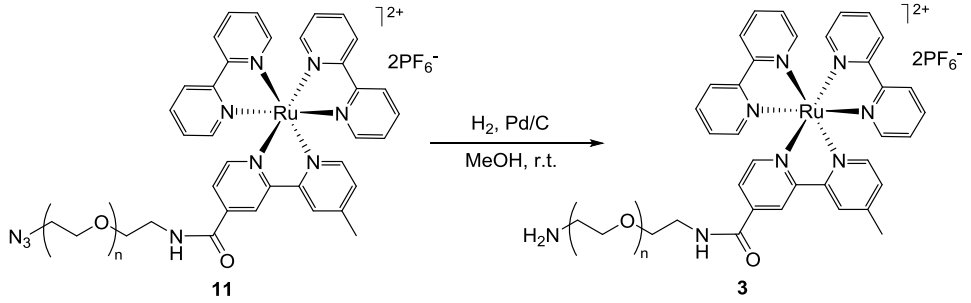
N_3-PEG300-Ru(bpy)$_3$2PF$_6$ (11). To a solution of **9** (100 mg, 0.109 mmol), EDCI·HCl (42.3 mg, 0.238 mmol) and HOBt (31.1 mg, 0.230 mmol) in 5 mL of DMF was added **10** (124 mg, 0.404 mmol) and DIEA (32.3 mg, 0.250 mmol) under Ar. After stirring at room temperature for 17 h, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in 5% MeCN/H_2O and

purified with reverse-phase column chromatography (5-40% MeCN/H_2O), and compound **11** was yield as red amorphous solid (119 mg, 88% yield).

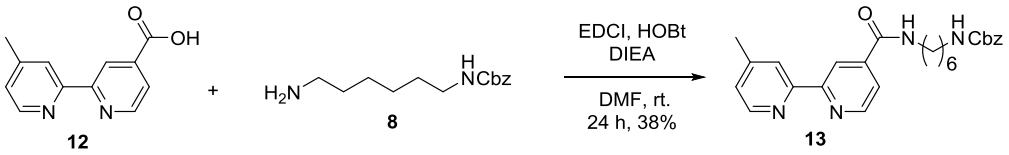
^{1}H NMR (500 MHz, CD_3CN) δ 8.93-8.86 (m, 1H), 8.60-8.56 (m, 1H), 8.50 (d, *J* = 8.0 Hz, 4H), 8.06 (t, *J* = 8.0 Hz, 4H), 7.84 (d, *J* = 5.5 Hz, 1H), 7.74-7.67 (m, 5H), 7.55 (d, *J* = 6.0 Hz, 1H), 7.41-7.37 (m, 4H), 7.27 (d, *J* = 5.5 Hz, 1H), 3.62- 3.46 (m, 28H), 3.34-3.30 (m, 2H), 2.55 (s, 3H); ^{13}C NMR (125 MHz, CD_3CN) δ 158.9, 157.9, 157.8, 157.7, 157.0, 153.2, 152.7, 152.6, 152.5, 151.7, 143.5, 138.8, 129.6, 128.6, 128.5, 126.6, 125.6, 125.5, 125.2, 122.6, 71.0, 70.9, 70.8, 70.3, 69.9, 69.8, 51.4, 40.9, 21.2; FT-IR (KBr) 3432, 3114, 3081, 2871, 2102, 1666, 1620, 1604, 1543, 1446, 1305, 1239, 1104 cm^{-1}; HRMS (ESI, positive): *m/z* calced. for $C_{44}H_{50}N_{10}O_6Ru$ (n=5) $[M]^{2+}$: 458.1480, found 458.1489. See Figure S13 for ESI-TOF MS spectrum data.



Bis(2,2'-bipyridine)[*N*-(6-aminohexyl)-4'-methyl-(2,2'-bipyridine)-4-carboxamide]ruthenium (II) bis(hexafluorophosphate) (2). To a solution of **6** (2.0 mg, 1.9 μmol) in 0.5 mL of MeOH was added 3.0 mg of 10% Pd/C (50% moisture content). After stirring under H_2 at room temperature for 7 h, Pd/C was removed by Celite filtration and the filtrate was concentrated under the reduced pressure to give **2** as a red solid. The red solid was applied next step directly without further purification.

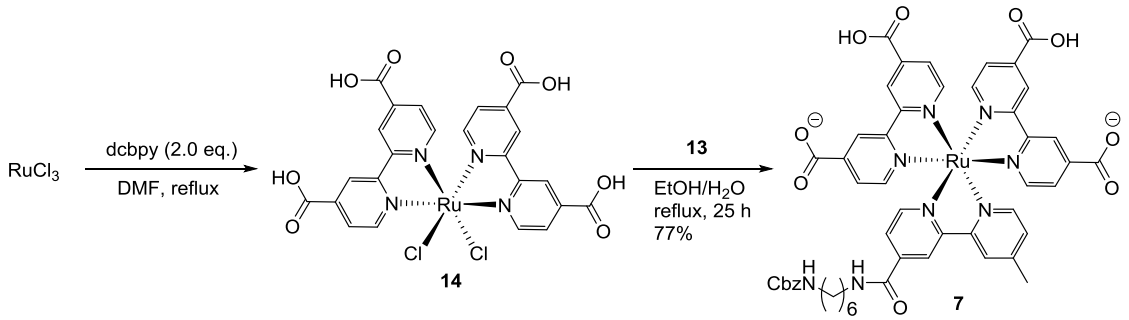


H_2N--PEG300-Ru(bpy)$_3$2PF$_6$ (3). To a solution of **11** (10.0 mg, 8.0 μmol) in 2.0 mL of MeOH was added 3.0 mg of 10% Pd/C (50% moisture content). After stirring under H_2 at room temperature for 2 h, Pd/C was removed by Celite filtration and the filtrate was concentrated under the reduced pressure to give **7** as a red solid. The red amorphous solid was applied next step directly without further purification.



Benzyl[6-(4-methyl-2,2'-bipyridyl-4'-carboxyamido)hexyl]carbamate (13). To a solution of **12** (50.2 mg, 0.239 mmol), EDCI·HCl (59.1 mg, 0.308 mmol), HOBt (39.4 mg, 0.292 mmol) and DIEA (90.0 mg, 0.696 mmol) in 2.0 mL of DMF was added **8** (83.4 mg, 0.291 mmol) under Ar. The reaction mixture was stirred at room temperature for 24 h. After addition of EtOAc and H_2O, the organic layer was collected, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography with CH_2Cl_2:MeOH=10:1 afforded **13** a white solid (20.8 mg, 25% yield).

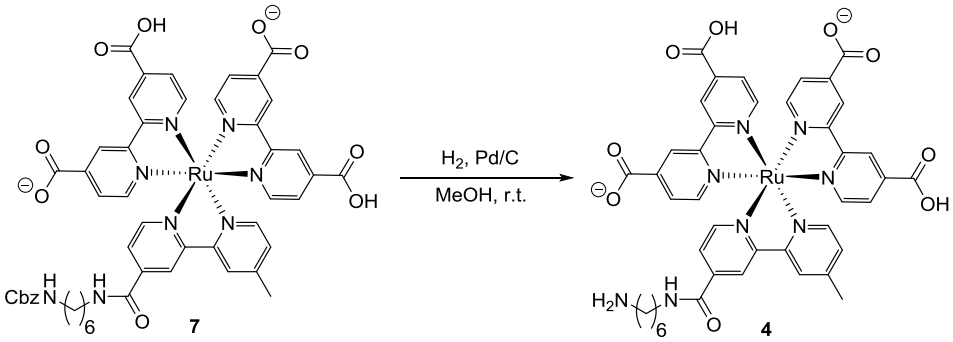
Mp 136-138 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.97 (d, J = 5.0 Hz, 1H), 8.62 (s, 1H), 8.53 (d, J = 5.0 Hz, 1H), 8.27 (s, 1H), 7.78 (dd, J = 2.0, 5.0 Hz, 1H), 7.35-7.29 (m, 5H), 7.18 (d, J = 4.0 Hz, 1H), 6.72 (brs, 1H), 5.09 (s, 2H), 4.79 (brs, 1H), 3.46 (q, J = 6.6 Hz, 2H), 3.12 (q, J = 6.8 Hz, 2H), 2.46 (s, 3H) 1.65-1.61 (m, 2H), 1.54-1.51 (m, 2H), 1.42-1.38 (m, 4H); ^{13}C NMR (125 MHz, CD_3CN) 165.8, 157.0, 156.7, 155.3, 150.3, 149.1, 148.7, 143.0, 136.8, 128.7, 128.2, 125.4, 122.4, 122.0, 117.5, 66.8, 40.8, 40.0, 30.0, 29.8, 29.5, 26.4, 26.1, 21.4; FT-IR (neat) 3329, 3286, 3034, 2939, 2870, 1683, 1631, 1595, 1530, 1478, 1342, 1283, 1255, 1221, 1142, 1100 cm^{-1}; HRMS (ESI, positive): *m/z* calced. for $C_{26}H_{31}N_4O_3$ $[M+H]^+$: 447.2391, found 447.2383.



Bis(4,4'-dicdicarboxy-2,2'-bipyridine){Benzyl[6-(4-methyl-2,2'-bipyridyl-4'-carboxyamido) hexyl]carbamate}ruthenium (II) bis-(hexafluorophosphate) (7). To a solution of $RuCl_3$ (207 mg, 1.00 mmol) in 5.0 mL of DMF was added **16** (487 mg, 2.00 mmol) under Ar. After stirring at 170 °C for 3 h, to the reaction mixture was added aqueous HCl solution (1 M). This mixture was cooled to 0 °C for 30 min and black precipitation was collected by filtration. The black precipitation was washed several times with 10 mL of aqueous HCl solution (1 M) three times and 5.0 mL of Et_2O three times, then dried *in vacuo* to give black solid **14**. Then, to a solution of **14** (92.1 mg, 0.139 mmol) in 8.0 mL of 75% EtOH/H_2O was added **13** (40.0 mg, 0.0925 mmol) under Ar. After stirring

under reflux for 25 h, the reaction mixture was concentrated *in vacuo*. The residue was purified with reverse-phase column chromatography (0-20% MeCN/H_2O) to give **7** as red solid (73.2 mg, 77% yield).

Mp >300 °C; ^{1}H NMR (500 MHz, D_2O) δ 8.86 (d, *J* = 7.5 Hz, 4H), 8.78 (s, 1H), 8.37 (s, 1H), 7.90 (d, *J* = 5.5 Hz, 1H), 7.85 (d, *J* = 5.5 Hz, 1H), 7.81 (t, *J* = 5.3 Hz, 2H), 7.78 (d, *J* = 5.5 Hz, 1H), 7.69-7.66 (m, 4H), 7.63 (d, *J* = 5.5 Hz, 1H), 7.57 (d, *J* = 6.0 Hz, 1H), 7.24 (d, *J* = 6.0 Hz, 1H), 6.96-6.90 (m, 5H), 4.60 (s, 2H), 3.15 (brs, 2H), 2.78 (brs, 2H), 2.39 (s, 3H) 1.34-1.32 (m, 2H), 1.13-1.11 (m, 2H), 0.97 (brs, 4H); ^{13}C NMR (125 MHz, D_2O) 170.6, 170.5, 170.3, 170.1, 165.3, 157.8, 157.6, 157.2, 157.1, 157.0, 155.3, 152.1, 151.9, 151.7, 151.5, 151.4, 151.0, 150.6, 145.7, 145.6, 141.9, 136.2, 128.9, 128.3, 127.9, 127.1, 126.4, 126.3, 125.3, 124.4, 123.2, 121.3, 66.0, 40.1, 28.7, 28.2, 25.7, 25.4, 20.5; FT-IR (KBr) 3329, 3286, 3067, 2938, 2870, 1683, 1629, 1608, 1541, 1370, 1267, 1137 cm^{-1}; HRMS (ESI, positive): *m/z* calced. for $C_{50}H_{42}Na_4N_8O_{11}Ru$ $[M+4Na]^{2+}$: 562.0805, found 562.0805. See Figure S14 for UV-Vis absorbance.



Bis(4,4'-dicarboxy-2,2'-bipyridine)[*N*-(6-aminohexyl)-4'-methyl-(2,2'-bipyridine)-4-carboxamide]ruthenium(II) bis(hexafluorophosphate) (4). To a solution of **7** (2.0 mg, 1.9 μmol) in 1.0 mL of MeOH was added 3.4 mg of 10% Pd/C (50% moisture content). After stirring for 10 h under H_2 at room temperature, Pd/C was removed by Celite filtration and the filtrate was concentrated under the reduced pressure to give **4** as a red solid. The red solid was applied next step directly without further purification.

2. Supporting fiures

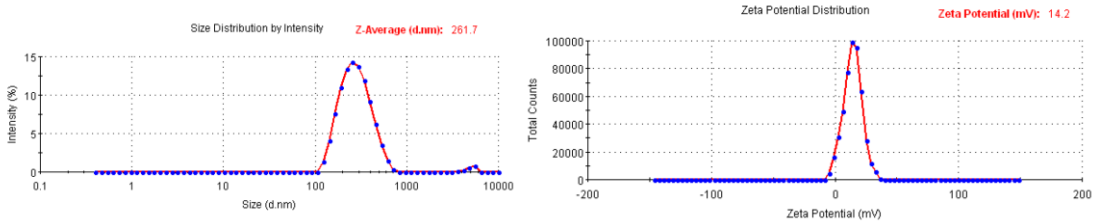


Figure S1. Size (left) and zeta-potential (right) of control FG beads

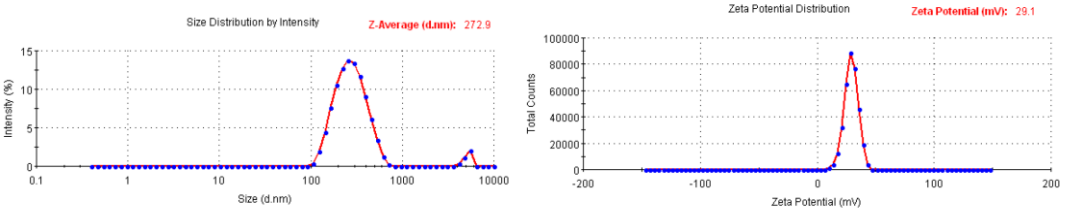


Figure S2. Size (left) and zeta-potential (right) of FG beads immobilized with **2**

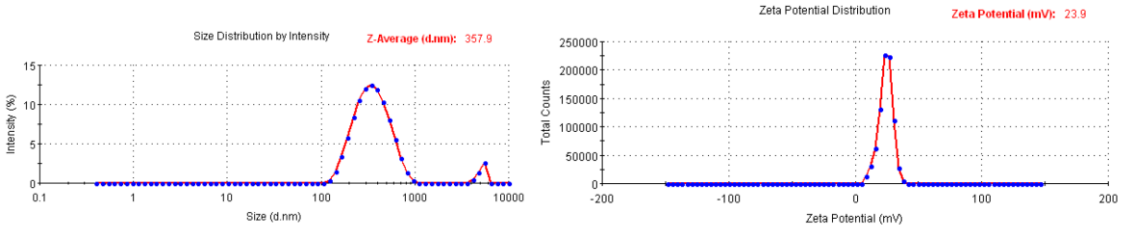


Figure S3. Size (left) and zeta-potential (right) of FG beads immobilized with **3**

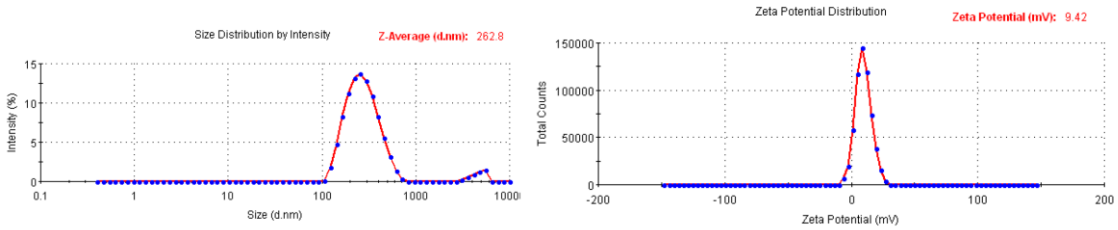


Figure S4. Size (left) and zeta-potential (right) of FG beads immobilized with **4**

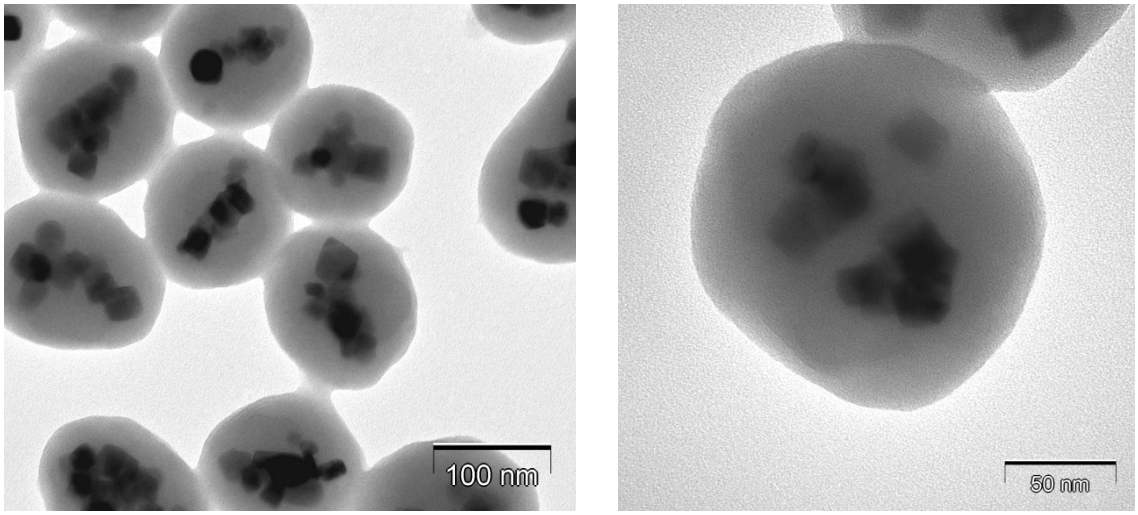


Figure S5. Transmission electron microscope images of FG beads immobilized with **4**

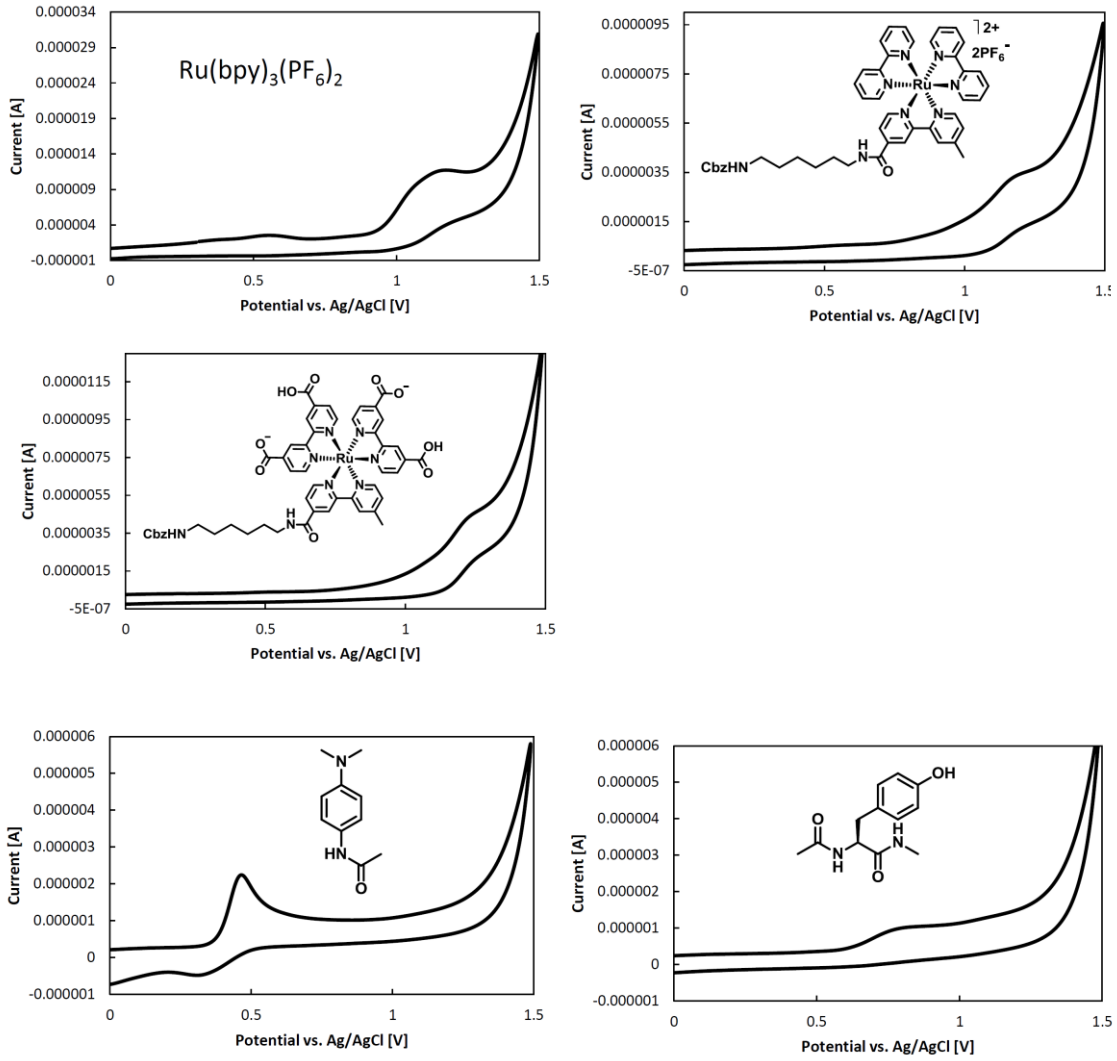
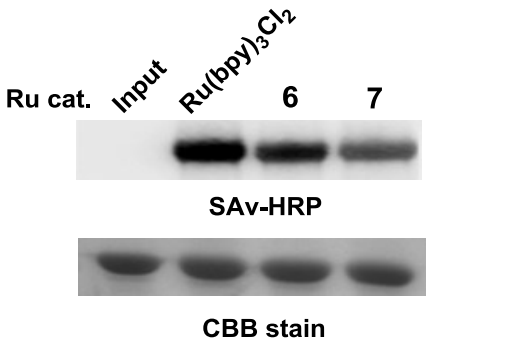
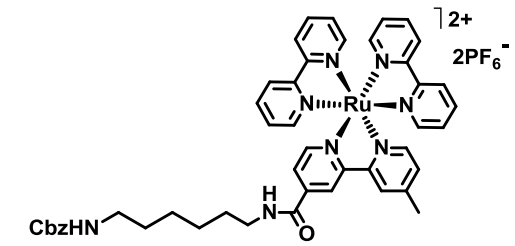


Figure S6. Oxidation potential of ruthenium complexes





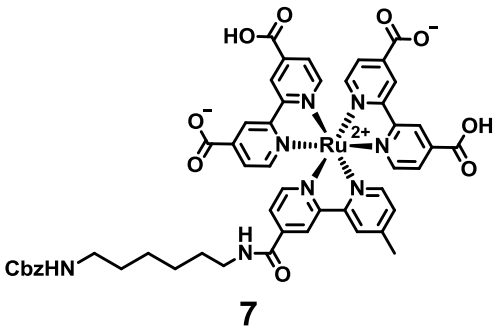
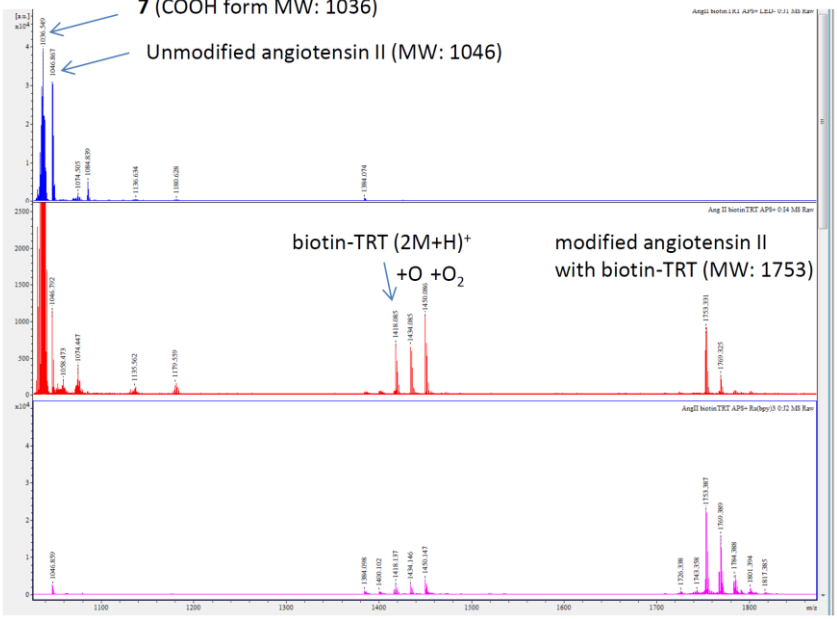


Figure S7. Labeling of bovine serum albumin using biotin-TRT and $Ru(bpy)_3Cl_2$, **6**, or **7**

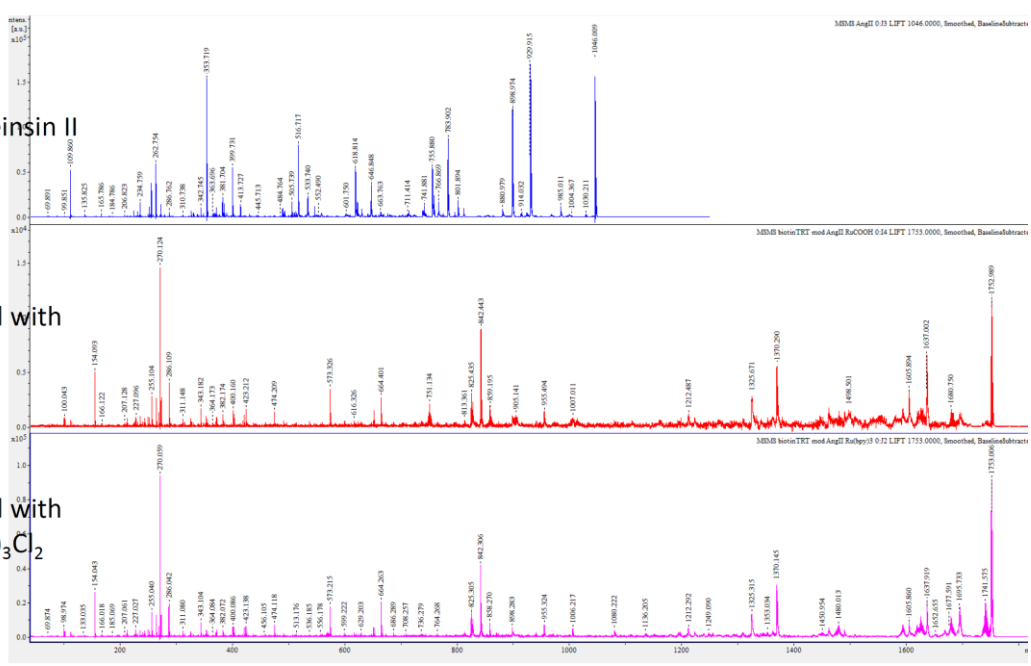
A)

Angiotensin II (DRVYIHPF), biotin-TRT

catalyst	LED irrad.
7	-
7	+
$Ru(bpy)_3Cl_2$	+

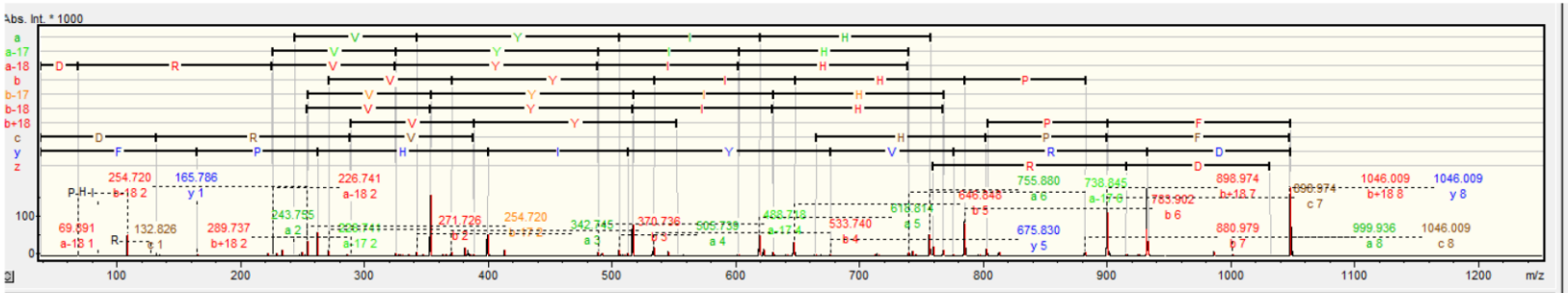


B)



C)

Angioteinsin II



Protein View | Match Errors | MS/MS Fragments | MSMS Analysis

Sequence: DRVYIHPF (Mods:)

MH+(mono): 1046.542 MH+(avg): 1047.188 MS/MS Tol: 0.800 Da Peaks: 111 Above Threshold: 111 Zoom to m/z for 2+ 1+ Zoom +/-: 10.0 Da

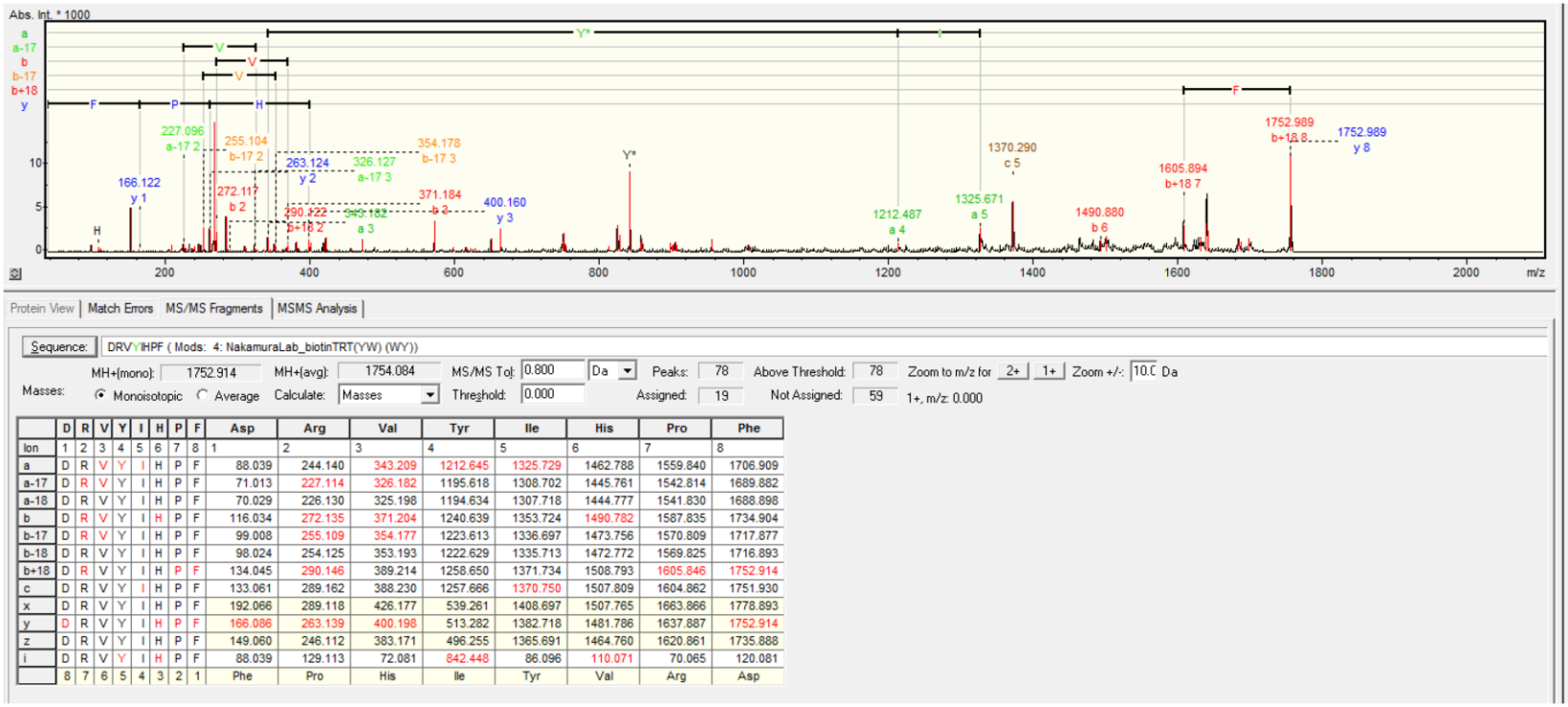
Masses: Monoisotopic Average Calculate: Masses Threshold: 0.000 Assigned: 45 Not Assigned: 66 1+, m/z: 0.000

	D	R	V	Y	I	H	P	F	Asp	Arg	Val	Tyr	Ile	His	Pro	Phe
Ion	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
a	D	R	V	Y	I	H	P	F	88.039	244.140	343.209	506.272	619.356	756.415	853.468	1000.536
a-17	D	R	V	Y	I	H	P	F	71.013	227.114	326.182	489.246	602.330	739.389	836.441	983.510
a-18	D	R	V	Y	I	H	P	F	70.029	226.130	325.198	488.262	601.346	738.405	835.457	982.526
b	D	R	V	Y	I	H	P	F	116.034	272.135	371.204	534.267	647.351	784.410	881.463	1028.531
b-17	D	R	V	Y	I	H	P	F	99.008	255.109	354.177	517.241	630.325	767.383	864.436	1011.505
b-18	D	R	V	Y	I	H	P	F	98.024	254.125	353.193	516.257	629.341	766.399	863.452	1010.521
b+18	D	R	V	Y	I	H	P	F	134.045	290.146	389.214	552.278	665.362	802.421	899.473	1046.542
c	D	R	V	Y	I	H	P	F	133.061	289.162	388.230	551.294	664.378	801.437	898.489	1045.558
x	D	R	V	Y	I	H	P	F	192.066	289.118	426.177	539.261	702.325	801.393	957.494	1072.521
y	D	R	V	Y	I	H	P	F	166.086	263.139	400.198	513.282	676.345	775.414	931.515	1046.542
z	D	R	V	Y	I	H	P	F	149.060	246.112	383.171	496.255	659.319	758.387	914.488	1029.515
i	D	R	V	Y	I	H	P	F	88.039	129.113	72.081	136.076	86.096	110.071	70.065	120.081
	8	7	6	5	4	3	2	1	Phe	Pro	His	Ile	Tyr	Val	Arg	Asp

D)

Labeled with

7



Protein View | Match Errors | MS/MS Fragments | MSMS Analysis

Sequence: DRVYIHPF (Mods: 4: NakamuraLab_biotinTRT(YW) (WY))

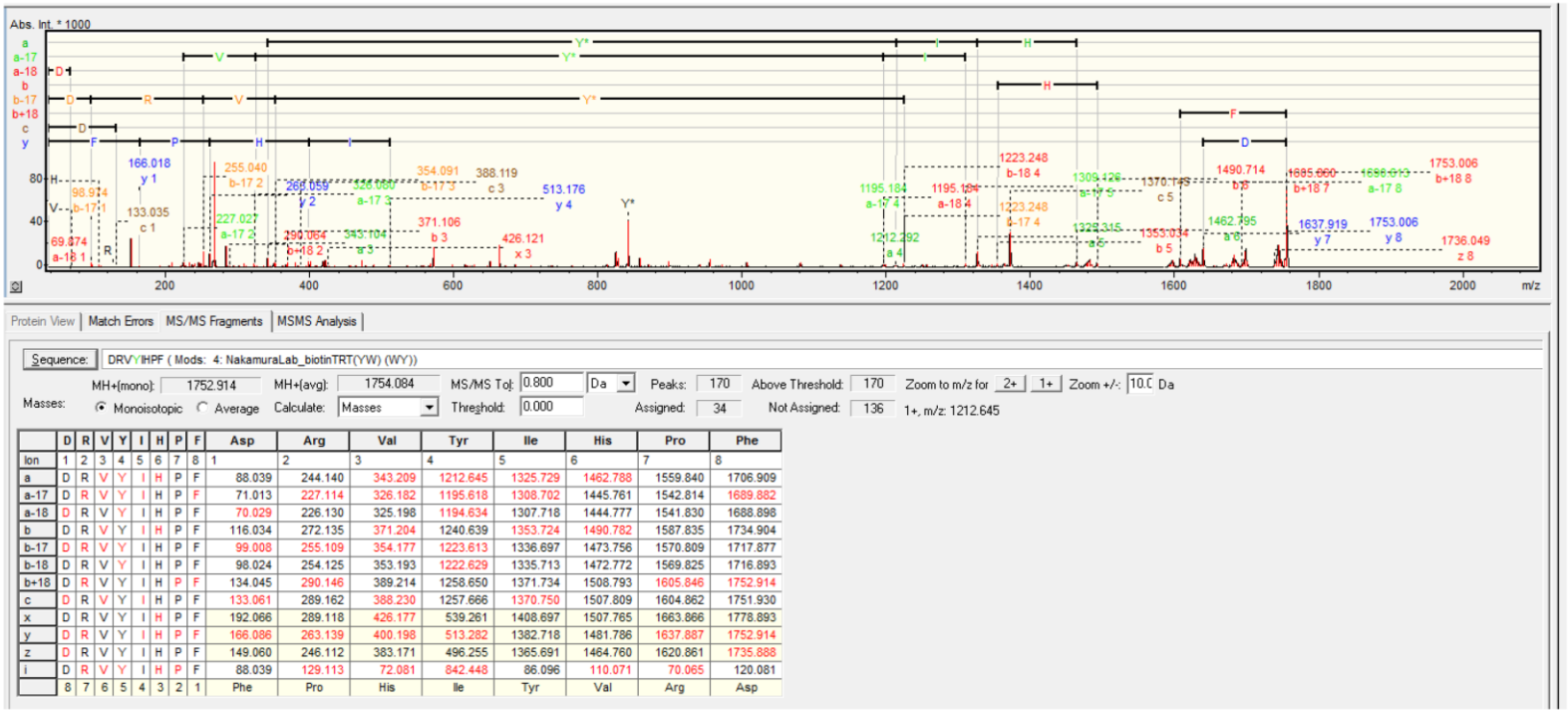
MH+(mono): 1752.914 MH+(avg): 1754.084 MS/MS Tol: 0.800 Da Peaks: 78 Above Threshold: 78 Zoom to m/z for 2+ 1+ Zoom +/-: 10.0 Da

Masses: Monoisotopic Average Calculate: Masses Threshold: 0.000 Assigned: 19 Not Assigned: 59 1+, m/z: 0.000

	D	R	V	Y	I	H	P	F	Asp	Arg	Val	Tyr	Ile	His	Pro	Phe
Ion	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
a	D	R	V	Y	I	H	P	F	88.039	244.140	343.209	1212.645	1325.729	1462.788	1559.840	1706.909
a-17	D	R	V	Y	I	H	P	F	71.013	227.114	326.182	1195.618	1308.702	1445.761	1542.814	1689.882
a-18	D	R	V	Y	I	H	P	F	70.029	226.130	325.198	1194.634	1307.718	1444.777	1541.830	1688.898
b	D	R	V	Y	I	H	P	F	116.034	272.135	371.204	1240.639	1353.724	1490.782	1587.835	1734.904
b-17	D	R	V	Y	I	H	P	F	99.008	255.109	354.177	1223.613	1336.697	1473.756	1570.809	1717.877
b-18	D	R	V	Y	I	H	P	F	98.024	254.125	353.193	1222.629	1335.713	1472.772	1569.825	1716.893
b+18	D	R	V	Y	I	H	P	F	134.045	290.146	389.214	1258.650	1371.734	1508.793	1605.846	1752.914
c	D	R	V	Y	I	H	P	F	133.061	289.162	388.230	1257.666	1370.750	1507.809	1604.862	1751.930
x	D	R	V	Y	I	H	P	F	192.066	289.118	426.177	539.261	1408.697	1507.765	1663.866	1778.893
y	D	R	V	Y	I	H	P	F	166.086	263.139	400.198	513.282	1382.718	1481.786	1637.887	1752.914
z	D	R	V	Y	I	H	P	F	149.060	246.112	383.171	496.255	1365.691	1464.760	1620.861	1735.888
i	D	R	V	Y	I	H	P	F	88.039	129.113	72.081	842.448	86.096	110.071	70.065	120.081
	8	7	6	5	4	3	2	1	Phe	Pro	His	Ile	Tyr	Val	Arg	Asp

E)

Labeled with
$Ru(bpy)_3Cl_2$



	D	R	V	Y	I	H	P	F	Asp	Arg	Val	Tyr	Ile	His	Pro	Phe
Ion	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
a	D	R	V	Y	I	H	P	F	88.039	244.140	343.209	1212.645	1325.729	1462.788	1559.840	1706.909
a-17	D	R	V	Y	I	H	P	F	71.013	227.114	326.182	1195.618	1308.702	1445.761	1542.814	1689.882
a-18	D	R	V	Y	I	H	P	F	70.029	226.130	325.198	1194.634	1307.718	1444.777	1541.830	1688.898
b	D	R	V	Y	I	H	P	F	116.034	272.135	371.204	1240.639	1353.724	1490.782	1587.835	1734.904
b-17	D	R	V	Y	I	H	P	F	99.008	255.109	354.177	1223.613	1336.697	1473.756	1570.809	1717.877
b-18	D	R	V	Y	I	H	P	F	98.024	254.125	353.193	1222.629	1335.713	1472.772	1569.825	1716.893
b+18	D	R	V	Y	I	H	P	F	134.045	290.146	389.214	1258.650	1371.734	1508.793	1605.846	1752.914
c	D	R	V	Y	I	H	P	F	133.061	289.162	388.230	1257.666	1370.750	1507.809	1604.862	1751.930
x	D	R	V	Y	I	H	P	F	192.066	289.118	426.177	539.261	1408.697	1507.765	1663.866	1778.893
y	D	R	V	Y	I	H	P	F	166.086	263.139	400.198	513.282	1382.718	1481.786	1637.887	1752.914
z	D	R	V	Y	I	H	P	F	149.060	246.112	383.171	496.255	1365.691	1464.760	1620.861	1735.888
i	D	R	V	Y	I	H	P	F	88.039	129.113	72.081	842.448	86.096	110.071	70.065	120.081
	8	7	6	5	4	3	2	1	Phe	Pro	His	Ile	Tyr	Val	Arg	Asp

Figure S8. MS analysis of biotin-TRT binding site. (A) MS image of antioteinsin II and labeled angiotensin II. Labeling condition: angioteinsin II (100 mM), biotin-TRT (0.5 mM), $Ru(bpy)_3Cl_2$ or **7** (1.0 mM), APS (1.0 mM) LED(455 nm, 5min) (B) Whole image of MS/MS analysis of antioteinsin II and labeled angiotensin II. The MS/MS fragment patterns of labeled peptides were similar. (C) Assign of MS/MS analysis for angitoteinsin II. The detected ions type *a*, *b*, *c*, *y* were well assigned sequences of angitotensin II. (D) Assign of MS/MS analysis for angitoteinsin II labeled with **7** and biotin-TRT. The detected ions type *a* strongly suggested the labeling at tyrosine residue. (E) Assign of MS/MS analysis for angitoteinsin II labeled with $Ru(bpy)_3Cl_2$ and biotin-TRT. The detected ions type *a* and *b* strongly suggested the labeling at tyrosine residue.

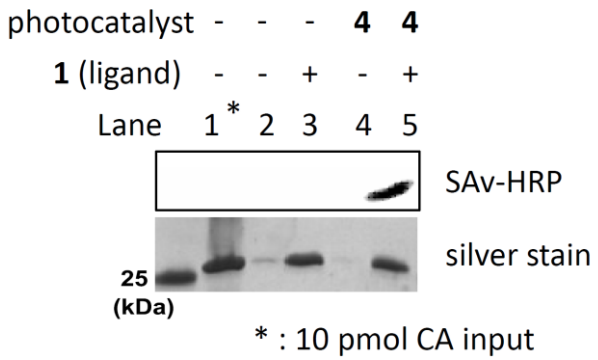


Figure S9. CA labeling with biotin-TRT after purification from protein mixture.

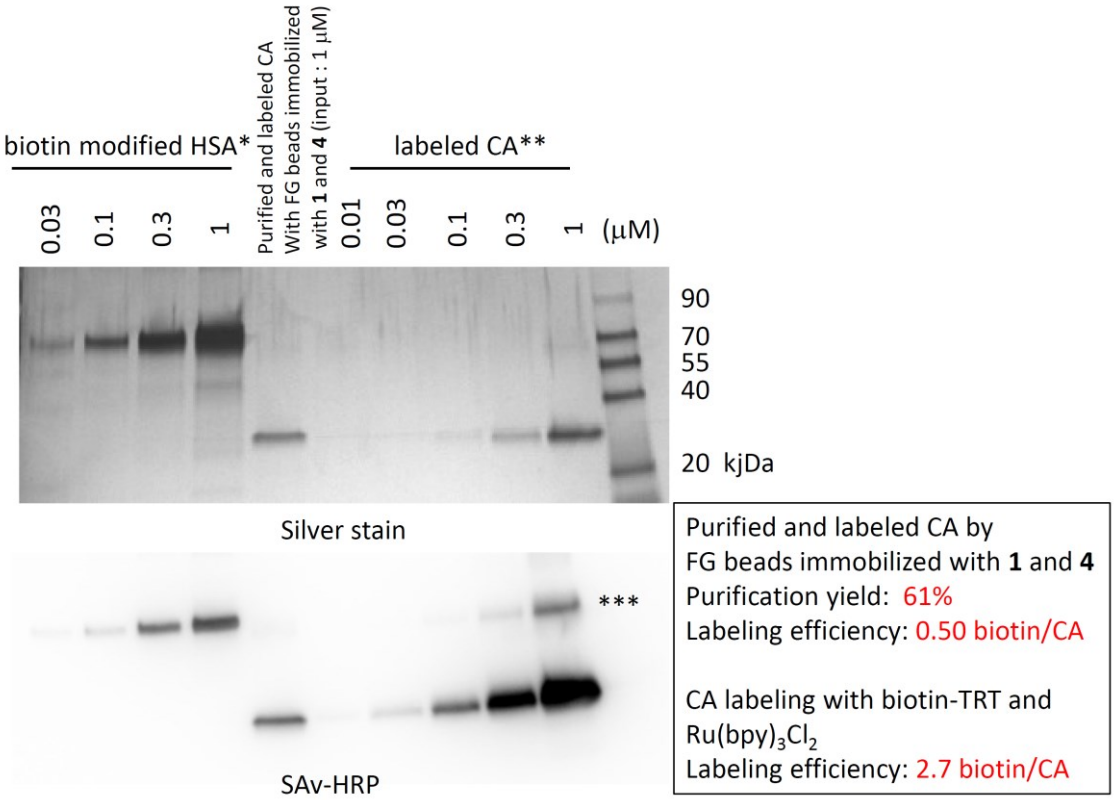


Figure S10. Analysis of purification efficiency and labeling efficiency

*HSA modified with maleimide-PEG4-biotin. This modified HSA containg 1.0 biotin per HSA at Cys34, and used as the control. **labeling condition :CA (1.0 μM) was mixed with $Ru(bpy)_3Cl_2$ (1.0 mM), biotin-TRT (0.5 mM) APS (1.0 mM) in 10 mM MES buffer (pH6.0) , and irradiated LED (455 nm, 5 min) on ice. ***Dimer of labeled CA.

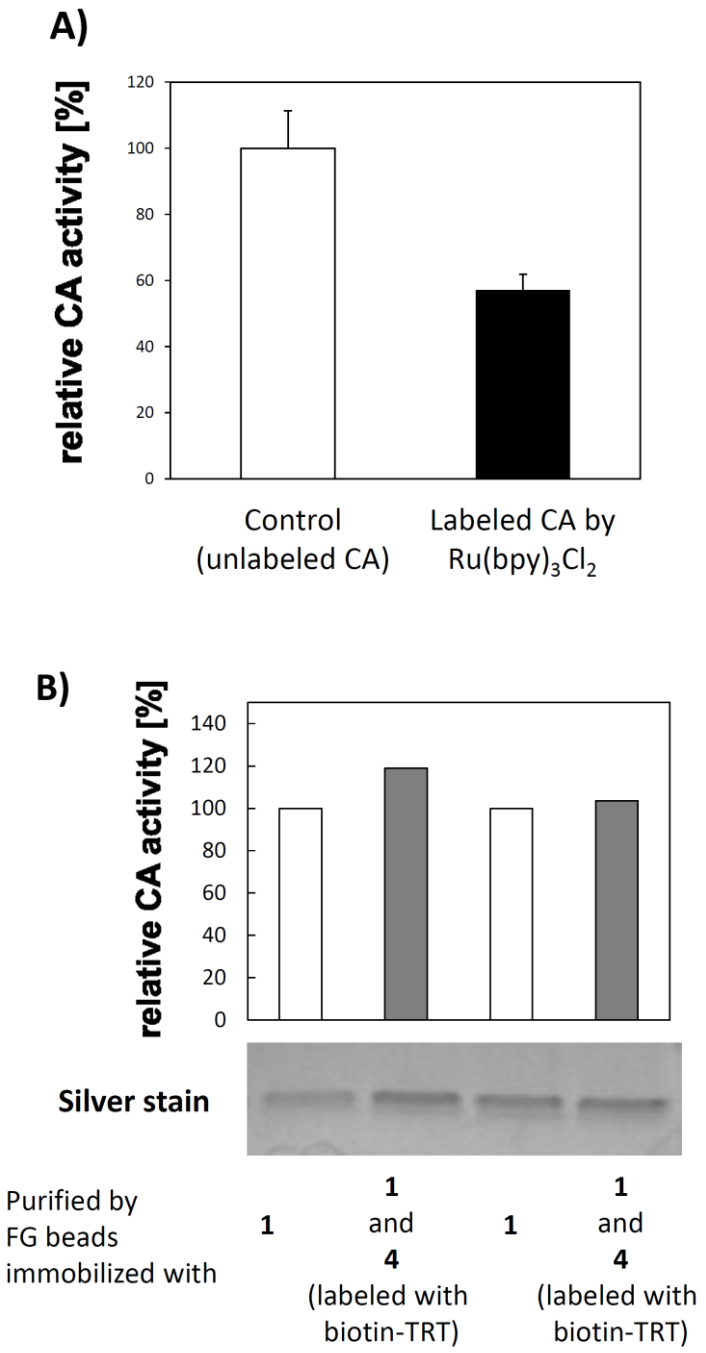


Figure S11. Enzymatic activity of labeled CA. (A) Enzymatic activity of CA labeled with $Ru(bpy)_3Cl_2$. The labeling condition was same as shown in Figure S10. Labeled CA (an average 2.7 molecules of biotin-TRT per CA molecule) showed 57% activity against control. (B) Enzymatic activity of CA purified and labeled with FG beads immobilized with **1** and **4**. Even after purification and labeling, labeled CA showed almost the same activity as CA purified with FG beads immobilized with **1**. The experiments were performed with duplicate.

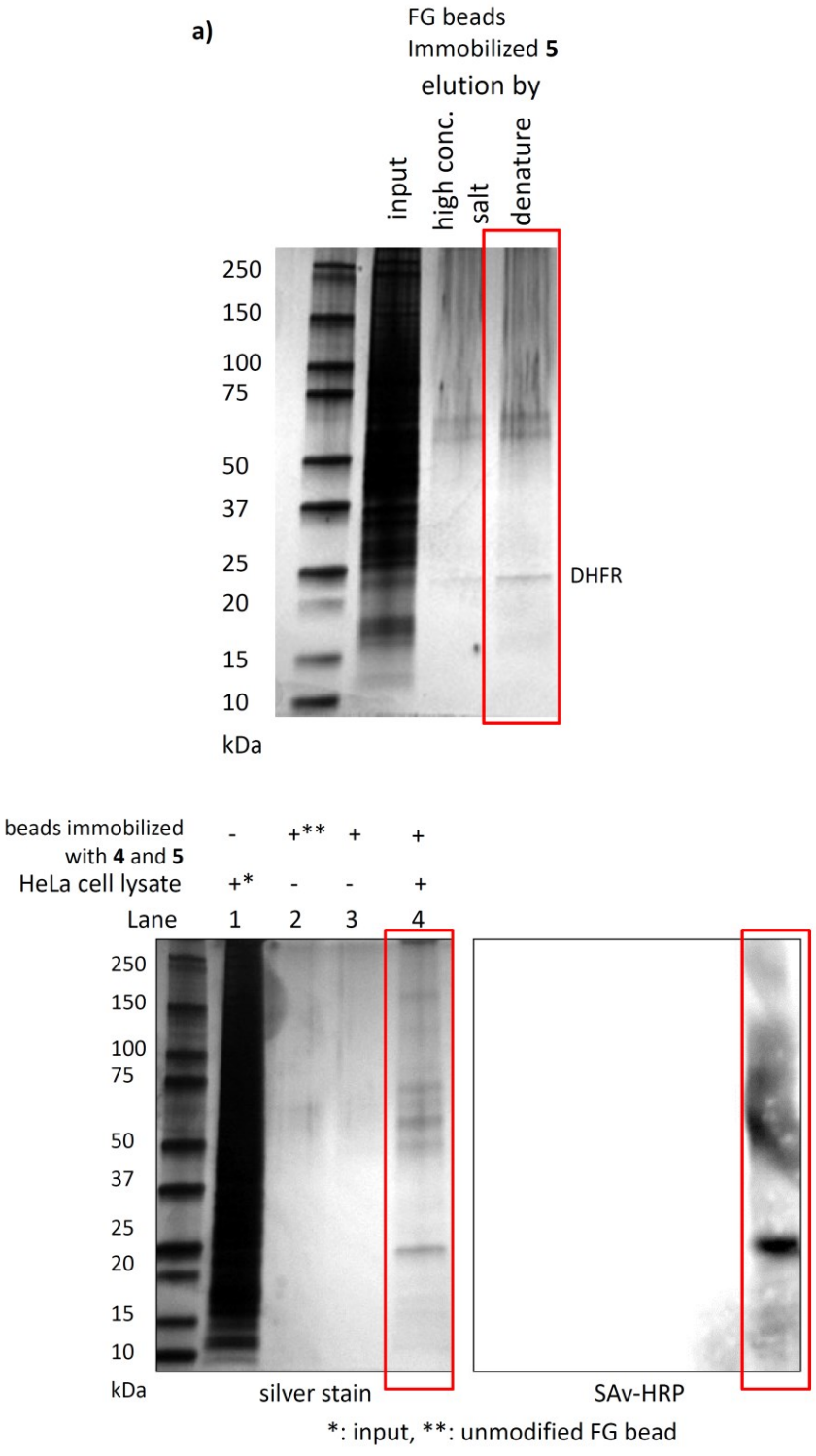


Figure S12. Whole PAGE images of Figure 4b. (a) Purification of DHFR using the FG beads immobilized with **5**. (b) Purification and labeling of DHFR using the FG beads immobilized with **4** and **5**. The part surrounded by red was used in Figure 4b.

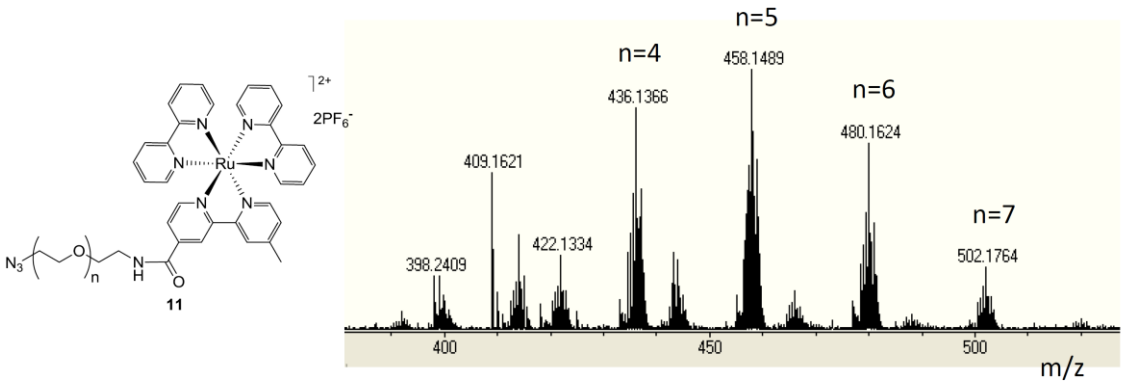


Figure S13. ESI-TOF MS spectrum of **11**.

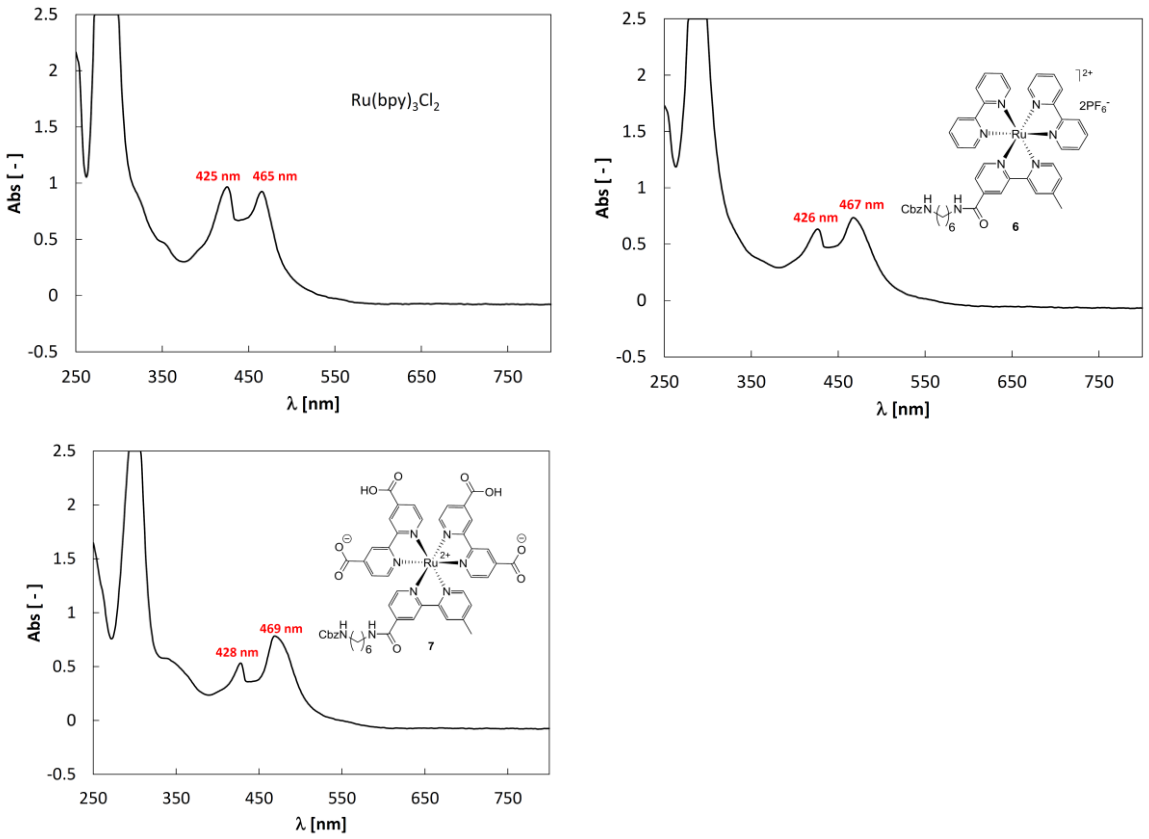
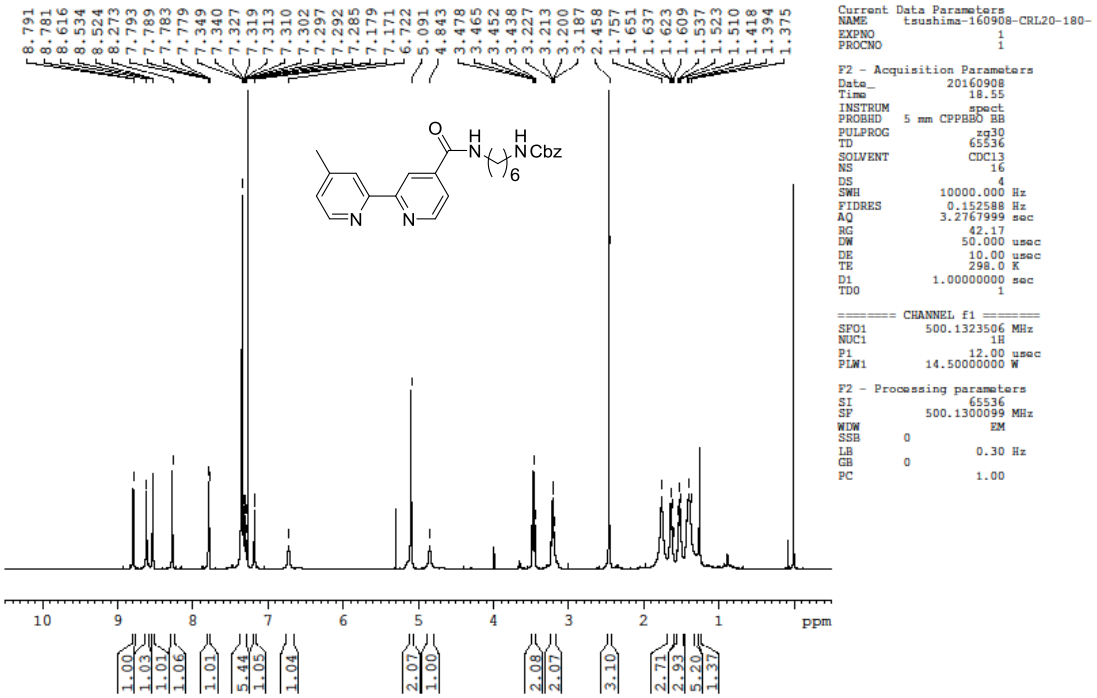
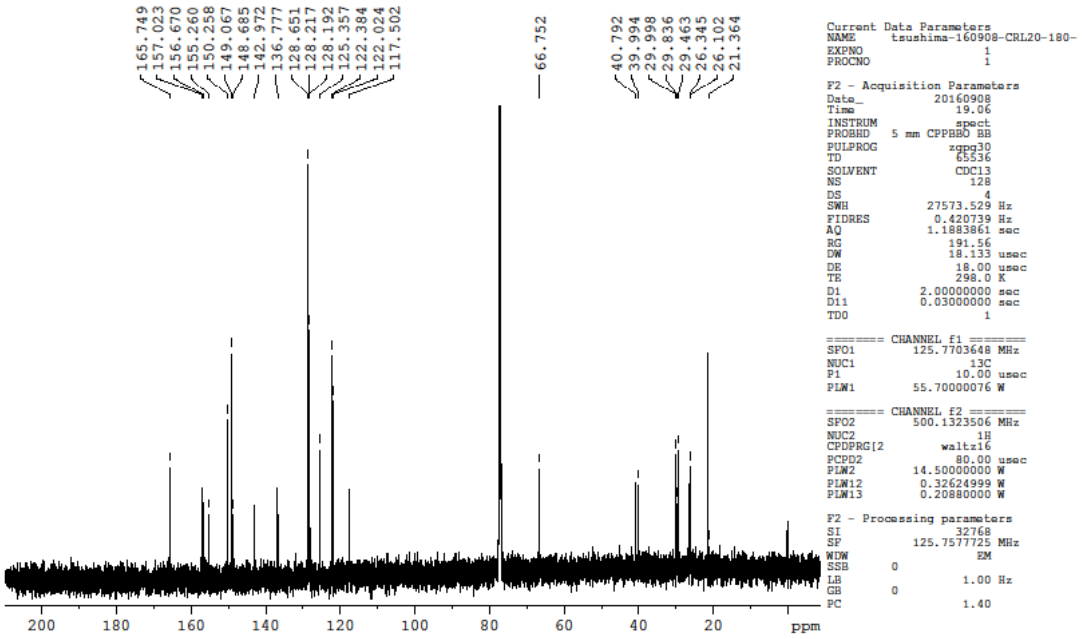
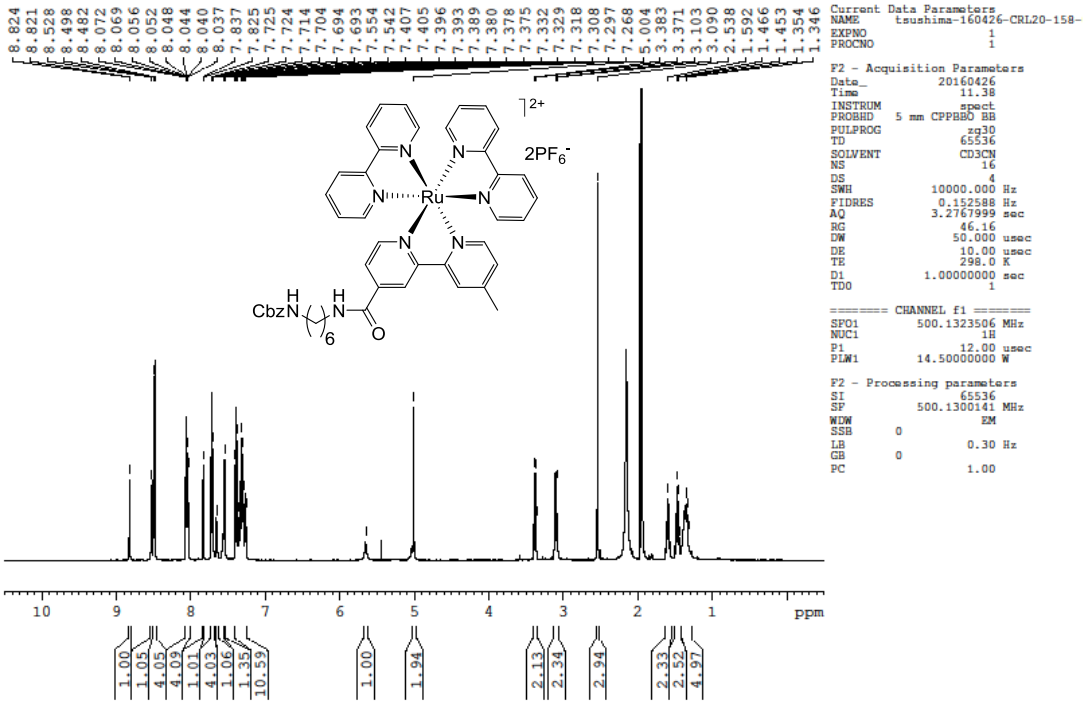


Figure S14. UV-Vis absorbance of $Ru(bpy)_3Cl_2$, **6** and **7**.

3. ^{1}H and ^{13}C NMR spectra of compounds





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RG                46.16
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DE                10.00 usec
TE                298.0 K
D1            1.00000000 sec
TD0                  1

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P1                12.00 usec
PLW1         14.50000000 W

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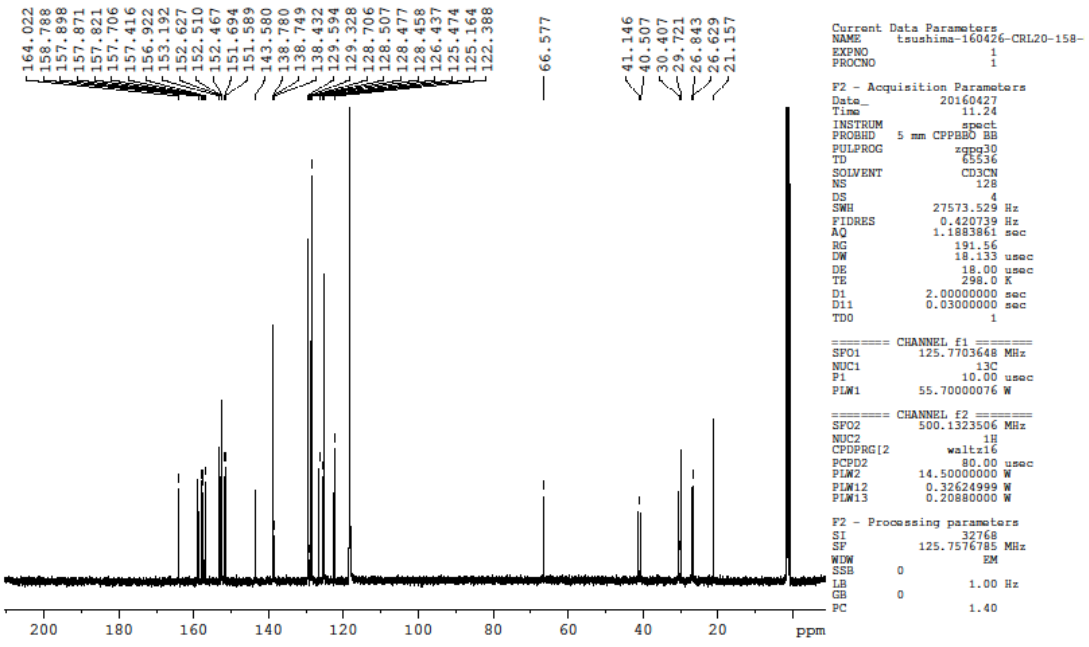


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TE                298.0 K
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D11           0.03000000 sec
TD0                  1

======== CHANNEL f1 ========
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NUC1               13C
P1                10.00 usec
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PCPD2             80.00 usec
PLW2         14.50000000 W
PLW12         0.32624999 W
PLW13         0.20880000 W

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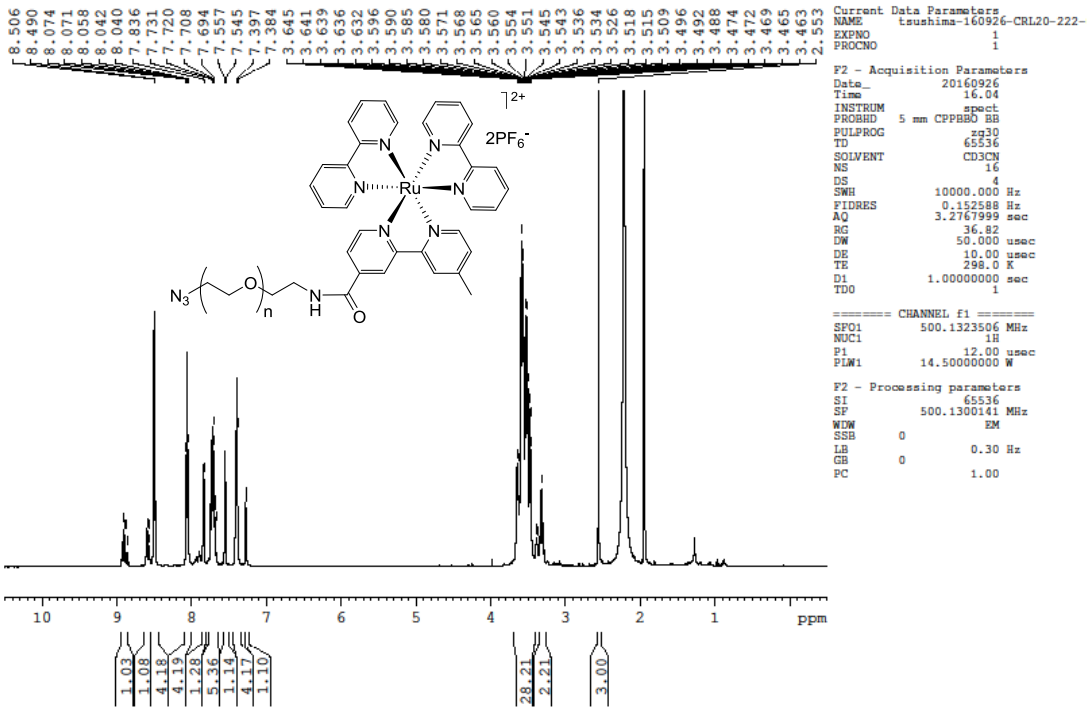
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2PF6-
Ru
N3
O
n
N
H
O
10 9 8 7 6 5 4 3 2 1 ppm
1.03 1.08 4.18 4.19 1.28 5.36 1.14 4.17 1.10 28.21 2.21 3.00
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DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
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NUC1 1H
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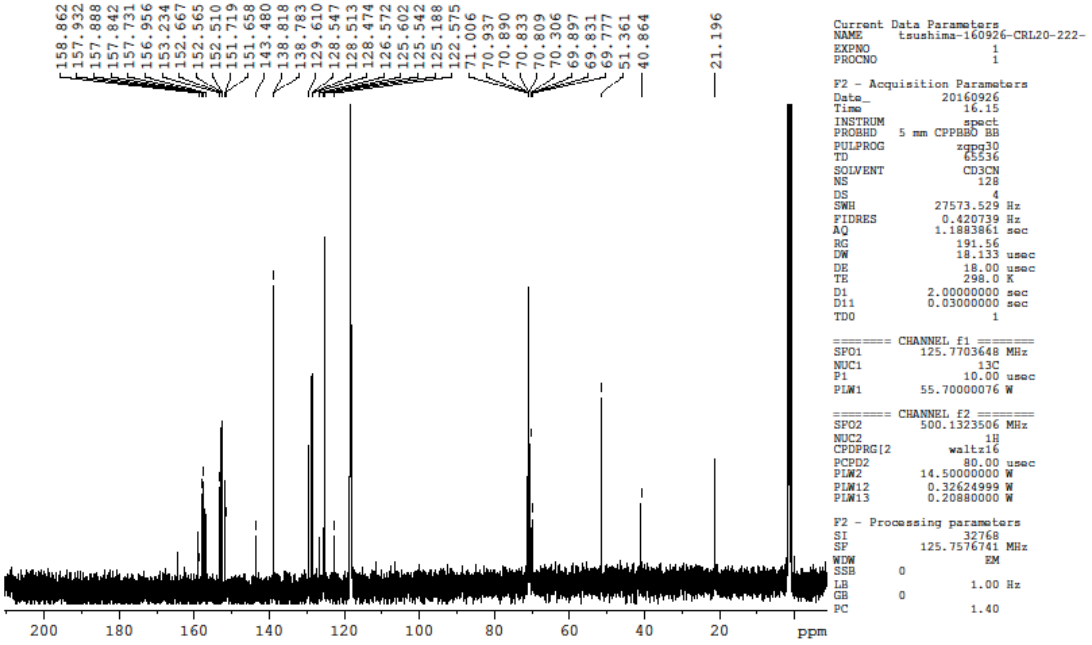
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RG 191.56
DW 18.133 usec
DE 18.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
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P1 10.00 usec
PLW1 55.70000076 W
======= CHANNEL f2 =======
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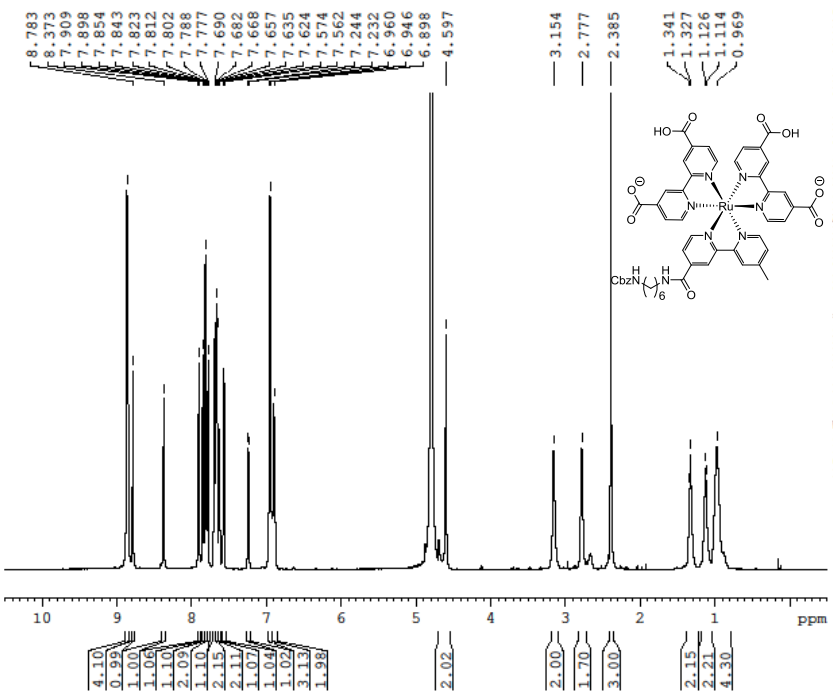


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TE                298.0 K
D1             1.00000000 sec
TD0                   1

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NUC1                  1H
P1                 12.00 usec
PLW1          14.50000000 W

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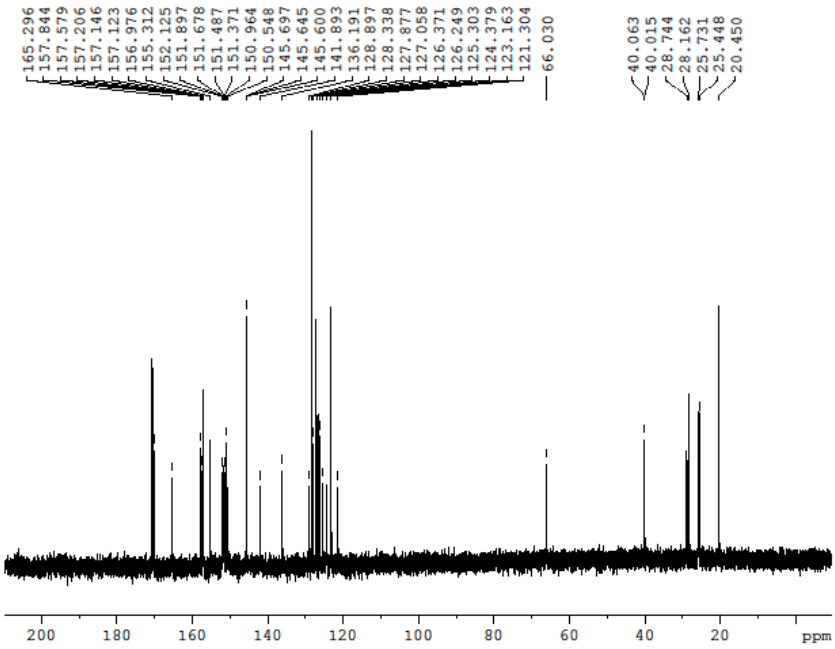


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PROCNO                1

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SWH           27573.529 Hz
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RG                191.56
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PCPD2              80.00 usec
PLW2          14.50000000 W
PLW12          0.32624999 W
PLW13          0.20880000 W

F2 - Processing parameters
SI                32768
SF            125.7577890 MHz
WDW                  EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

4. References

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