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Supporting Information

An Anthraquinone—Enzyme—Peptide Hybrid as a Photo-Switchable Enzyme

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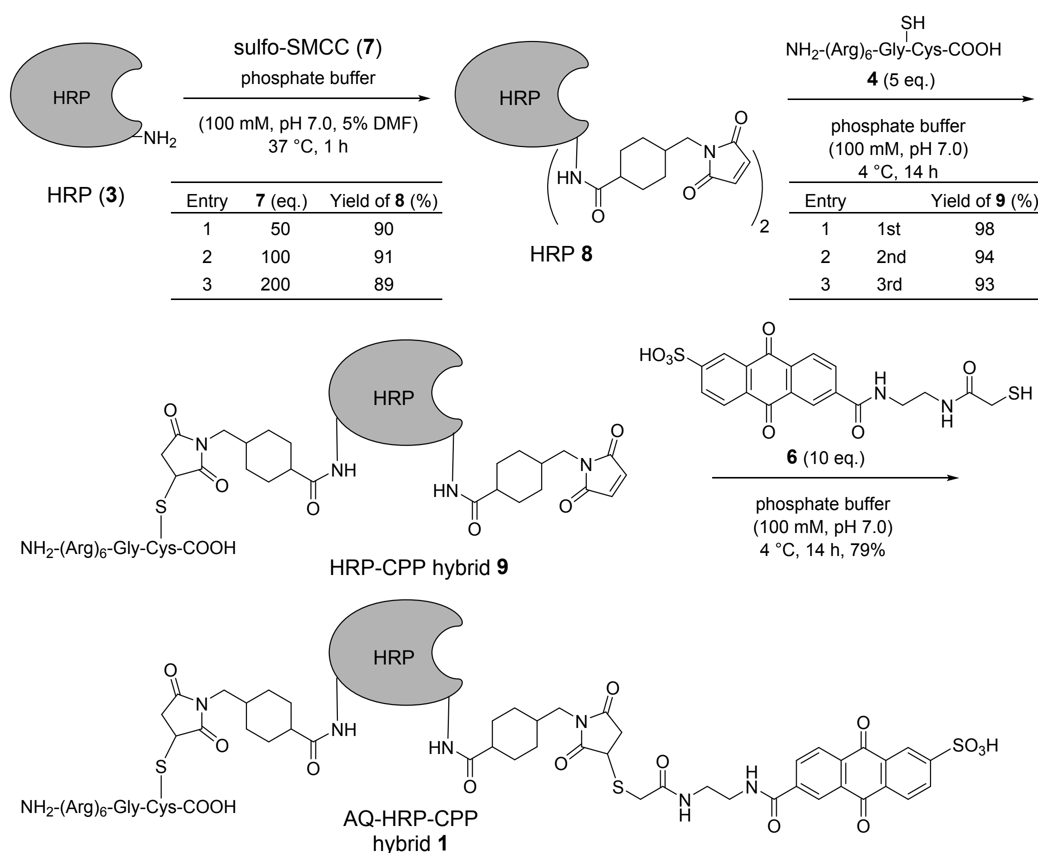
General methods for chemical synthesis

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS) was conducted using a Bruker Ultraflex mass spectrometer with detection in linear mode. Sinapinic acid was used as the matrix, with positive ionization mode. UV/vis spectra were recorded on a JASCO V-550 spectrometer.

Materials

Sulfo-SMCC (**7**) was purchased from Dojindo. Horseradish peroxidase (HRP) (**3**) was purchased from Roche. R_6GC peptide (**4**)[1] was purchased from Scrum. An antibody to α-tubulin (DM1A) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). An antibody to HRP (HP-03) was purchased from Abcam. HRP-conjugated anti-mouse IgG (NA931A) was purchased from GE Healthcare. The human epidermoid squamous carcinoma cell line, A431 (RCB0202), and the human normal diploid fibroblast cell line, WI-38 (RCB0704) were provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan.

Synthesis of AQ—HRP—CPP hybrid 1



Entry	7 (eq.)	Yield of 8 (%)
1	50	90
2	100	91
3	200	89

Entry		Yield of 9 (%)
1	1st	98
2	2nd	94
3	3rd	93

Scheme S1 Synthetic scheme of AQ—HRP—CPP hybrid **1**.

To a solution of HRP (**3**) (2.00 mg, 50.0 nmol) in 100 mM phosphate buffer (pH 7.0, 791 μL) was added sulfo-SMCC (**7**) (1.09 mg, 2500 nmol) in DMF (41.7 μL) at room temperature. After the reaction mixture was incubated for 1 h at 37 °C, Amicon 10K centrifugal filter device (Millipore) was used to separate from excess **7** and to concentrate the sample solution to give HRP—SMCC **8** (3.60 mg/mL, 500 μL, 90% yield).

To a solution of **8** (704 μg, 17.6 nmol) in 100 mM phosphate buffer (pH 7.0, 294 μL) was added R_6GC peptide **4** (98.3 μg, 88.0 nmol) in 100 mM phosphate buffer (pH 7.0, 98.3 μL) at room temperature. After the reaction mixture was incubated for 14 h at 4 °C, Amicon 10K centrifugal filter device was used to separate from excess **4** and to concentrate the sample solution to give HRP—CPP hybrid **9** (3.29 mg/mL, 210 μL, 98% yield).

To a solution of HRP—CPP hybrid **9** (182 μg, 4.55 nmol) in 100 mM phosphate buffer (pH 7.0, 132 μL) was added AQ derivative **6**[2] (20.4 μg, 45.5 nmol) in 100 mM phosphate buffer (pH 7.0, 20.4 μL) at room temperature. After the reaction mixture was incubated for 14 h at 4 °C, Amicon 10K centrifugal filter device was used to separate from excess **6** and to concentrate the sample solution to give AQ—HRP—CPP hybrid **1** (0.753 mg/mL, 191 μL, 79% yield).

The chemical yields of **8**, **9** and **1** were calculated based on UV spectroscopy, MALDI TOF MS and Bradford method as shown below.

MALDI TOF MS analysis

The sample (1.00 μL) was mixed with sinapinic acid (1.00 μL in 0.1% TFA in water: acetonitrile = 50:50) matrix. Analyses by MALDI TOF MS were performed in the positive ion mode.

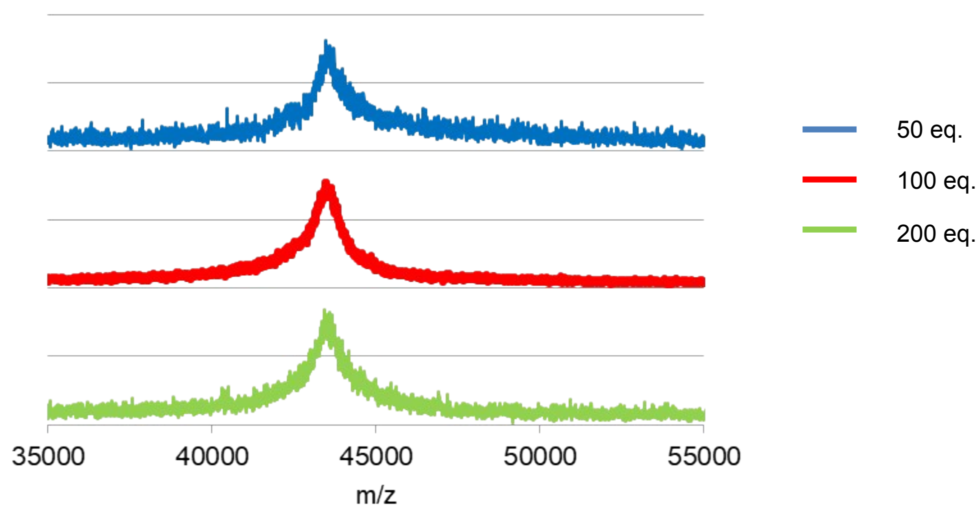


Fig. S1 MALDI-TOF MS spectra of HRP—SMCC **8** synthesized with **7** (50 eq.) (blue, M_w 43469), **7** (100 eq.) (red, M_w 43476) and **7** (200 eq.) (green, M_w 43418).

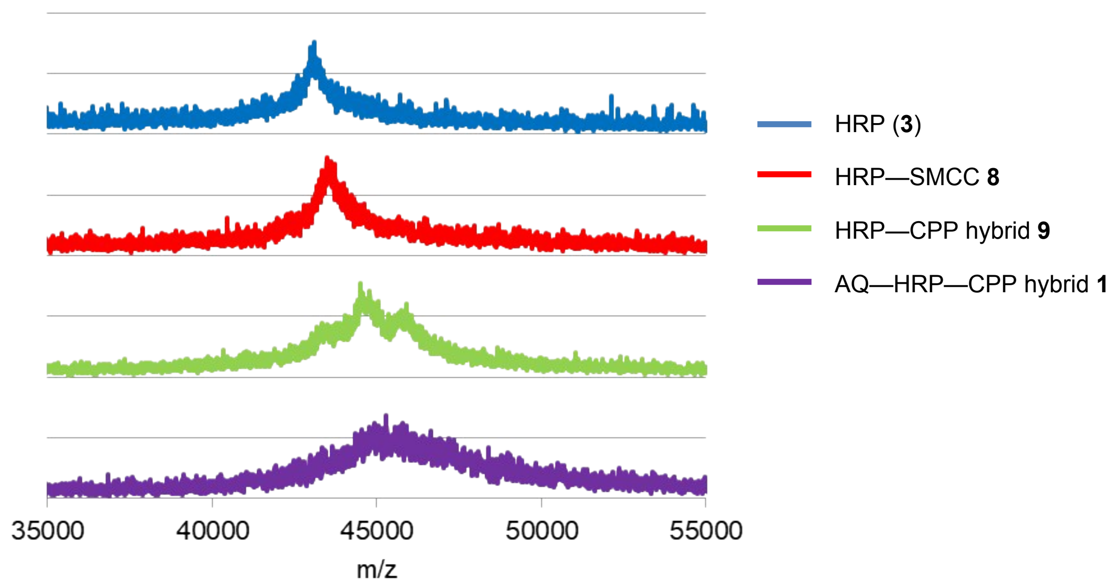


Fig. S2 MALDI-TOF MS spectra of HRP (**3**) (blue, M_w 42971), HRP—SMCC **8** (red, M_w 43469), HRP—CPP hybrid **9** (green, M_w 44486) and AQ—HRP—CPP hybrid **1** (purple, M_w 44925).

UV spectrum charts of hybrid 1 and HRP (3)

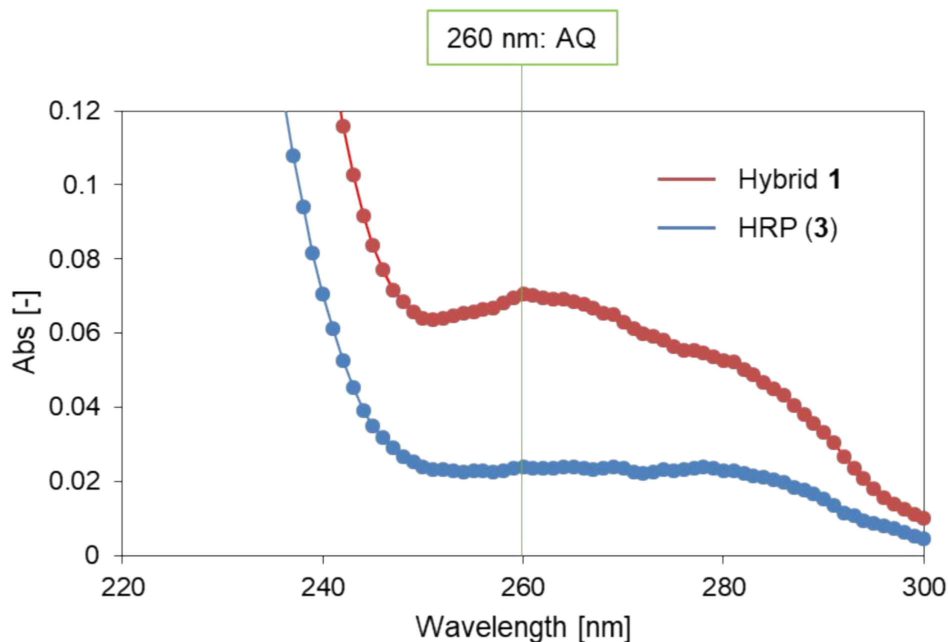


Fig. S3 UV spectra of **1** and **3**. These compounds (12 μM) were dissolved in phosphate buffer (100 mM, pH 7.0).

Bradford assay[3]

To a sample (10.0 μL) in test tube, a volume of 300 μL Bradford assay reagent (Thermo Scientific) was added, and then the resulting mixture was blended by gentle vortex mixing. After 5 min, absorbance at 595 nm was measured in plastic 96-well microplate against a reagent blank using microplate reader, SpectraMax i3 (Molecular Devices). The calibration curve was built using BSA samples (0.100-0.500 μg).

Photo-self-degradation of hybrid 1

A solution of HRP (**3**) or hybrid **1** (3 μM/lane) in 100 mM phosphate buffer (pH 7.0, 10.0 μL) was incubated at 25 °C for 0, 30, 60 and 120 min with or without photo-irradiation using a UV lamp (365 nm, 100 W) placed 10 cm from the sample. And then, 2.00 μL of electrophoresis buffer (Tris (0.35 M), SDS (10%, wt/vol), glycerol (36%, wt/vol), 2-mercaptoethanol (5%, wt/vol) and bromophenol blue (0.012%, wt/vol)) was added to the photo-irradiated samples. The photo-degradation products were separated by SDS-PAGE in 12.5% polyacrylamide gels. The gels were run by applying 30 mA for 90 min, stained with SYPRO Ruby Protein Gel Stain (Bio-Rad Lab. Inc.) for 14 h, destained in acetic acid (7%, vol/vol) and methanol (10%, vol/vol) for 0.5 h, and washed with deionized water. The gels were scanned with a Molecular Image FX (Bio-Rad Lab. Inc.). Molecular weight markers were used in each gel for calibration purpose.

Enzyme activity assay[4]

o-Phenylenediamine (OPD) stock solution (3.7 mM) was prepared by dissolving OPD in citrate buffer (25 mM citric acid, 58 mM sodium hydrogen phosphate, 2.65 mM H_2O_2). A solution of HRP (**3**) or hybrid **1** (2.5 nM) in citrate buffer (25 mM citric acid, 58 mM sodium hydrogen phosphate) was incubated at 25 °C for 10 min with or without photo-irradiation using a UV lamp (365 nm, 100 W) placed 10 cm from the sample. And then, the stock solution was added to the photo-irradiated samples. After the mixture was incubated for 10 min at 25 °C, 50.0 μL of 4 N H_2SO_4 solution was added to the samples to stop the enzymatic reaction. The absorbance at 490 nm was measured in plastic 96-well microplate using microplate reader.

Cell culture

< A431 cell>

The A431 cell line was routinely grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) Fetal bovine serum, 0.5% (v/v) penicillin and kanamycine. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 plus air.

<WI-38 cell>

The WI-38 cell line was routinely grown in Eagle's minimum essential medium (MEM) supplemented with 10% (v/v) Fetal bovine serum, 0.5% (v/v) penicillin and kanamycine. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 plus air.

Histological staining[5]

WI-38 or A431 cells (1×10^4 cells) in 100 μL of medium were cultured in 96-well microplates, and then incubated for 24 h at 37 °C under 5% CO_2 plus air. The cells were treated with a solution of HRP (**3**) or hybrid **1** (final concentration, 300 nM) in PBS (pH 7.4, 2 μL) for 24 h at 37 °C under 5% CO_2 plus air. The cells were washed with medium (3×100 μL), and then preincubated with or without photo-irradiation using a UV lamp (368 nm, 30 W) placed at 10 cm from the vessel at 25 °C for 3 h. After washing with PBS (3×100 μL), cells were fixed with 4% paraformaldehyde phosphate buffer solution (100 μL) for 15 min, washed again with PBS (1×100 μL). Cells were incubated with a staining solution of 3,3'-diaminobenzidine (DAB) (460 μM) and H_2O_2 (580 μM) in 10 mM PBS (pH 7.2, 100 μL) for 10 min. When the desired degree of staining intensity was reached, the reaction was terminated by washing in distilled water (2×100 μL). Images were collected on EVOS AMF-4302 microscope (Advanced Microsopy Group) using a 40x (transmitted light) objective.

Western blotting

WI-38 and A431 cells (5.0×10^5 cells) were plated on 60 mm dishes. After reaching confluence, cells were treated with a solution of HRP (**3**) or hybrid **1** (final concentration, 300 nM) for 24 h at 37 °C under 5% CO_2 plus air. The cells were washed three times with medium, and then preincubated with or without photo-irradiation using a UV lamp (368 nm, 30 W) placed at 10 cm from the dishes at room temperature for 3 h. After washing with cold PBS, adherent cells were scraped with rubber policeman and centrifuged for 5 min at 3500 rpm at 4 °C. The pellet was then resuspended in 50 μL lysis buffer (50 mM HEPES (pH 8.0), 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM PMSF, 1% triton X-100, 10% glycerol) containing protease inhibitor cocktail (NAKARAI TESQUE Inc.) and homogenized with ULTRA SONIC HOMOGENIZER UH-50 (SMT Co., Ltd). The lysate was centrifuged for 30 min at 13500 rpm at 4 °C. Equal amounts of protein were separated by SDS-PAGE in 10% polyacrylamide gels. Proteins were transferred onto nitrocellulose membranes Hybond™-ECL (GE Healthcare). Membranes were blocked with Tris-buffered saline-0.1% Tween 20 (TBST) containing 5% or 10% nonfat dry milk for 1 h at room temperature and membranes were incubated with appropriately diluted primary antibodies at 4 °C overnight. After washing five times with TBST, the blots were incubated with the horseradish peroxidase-conjugated specific secondary antibody for 2 h at 4 °C and then washed five times again. Then the complexes were visualized in Medical Film Processer FPM100 (Fujifilm Co.)

using the enhanced chemiluminescence reagents, Immobilon^{TM} Western (Millipore Co.). The following primary antibodies were used for detection of specific bands: horseradish peroxidase and α-tubulin. The following secondary antibodies were used for detection of specific bands: HRP-conjugated anti-mouse IgG.

MTT assay

WI-38 or A431 cells (1×10^4 cells) in 100 μL of medium were cultured in 96-well microplates, and then incubated for 24 h at 37 °C under 5% CO_2 plus air. The cells were treated with hybrid **1** (final concentration, 300 nM) in PBS (pH 7.4, 2 μL) for 24 h at 37 °C under 5% CO_2 plus air. The cells were washed with medium (3×100 μL), and then preincubated at 25 °C for 1, 2 and 3 h with or without photo-irradiation using a UV lamp (368 nm, 30 W) placed at 10 cm from the vessel. 10 μL of 5 mg/mL MTT dissolved in PBS was added to each well. After incubation for 3 h at 37 °C under 5% CO_2 plus air, 100 μL of DMSO was added to each well. The absorbance of the mixture was measured at 540 nm using microplate reader.

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