Electronic Supplementary Information

Photoresponsive Tandem Zinc Finger Peptide

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Experimental Procedure

Materials. All *N*-fluorenylmethoxycarbonyl (Fmoc)-protected amino acids and reagents used for an automatic peptide synthesizer were purchased from Applied Biosysytems. The oligodeoxynucleotide (ODN) containing GC box and AciI were purchased from Operon Biotechnologies and New England BioLabs, Inc., respectively. All other chemicals were of the highest commercial grade and were used without further purification.

Peptide synthesis and characterization. The peptide region of the azobenzene peptide shown in Figure 1 were synthesized on an automatic peptide synthesizer (Model 433A, Applied Biosysytems) using the Fmoc solid-phase method on an amide resin. To introduce the azobenzene unit to the peptide, Fmoc group at the N-terminus of the synthesized peptide was removed by 30% piperidine in *N*-methylpyrrolidone (NMP) on an automatic peptide synthesizer. Then, the peptide on resin was treated with 67 mM 4-azophenylbenzoyl chloride in the presence of 67 mM *N*-diisopropylethylamine (DIEA) in dimethylformamide (DMF) for 1 h at 25 °C to yield the azobenzene-containing protected peptide. The coupling success was verified by the Kaiser assay.[1] After the reaction, the resin was washed with DMF, methanol, and diethyl ether. The peptide was cleaved from the resin and deprotected by treatment with trifluoroacetic acid (TFA) / triisopropylsilane (TIS) / 1,2-ethanedithiol (EDT) / H_2O (94/1/2.5/2.5 v/v) at 20 °C for 1h. The solution containing free peptide was filtered, concentrated in vacuo, and precipitated with ether at 0 °C. The supernatant was decanted, and then the solid was washed with ether. The resultant solid was resuspended in acetic acid/H_2O (50/50, v/v) and lyophilized to yield a crude apo-peptide. Purification of the peptides was accomplished by reversed phase high-performance liquid chromatography (HPLC) on

Chemcobond 5-ODS-H (4.6 × 150 mm, Chemco Scientific Co., Ltd.) with a 30-min linear gradient of 20–50% acetonitrile/H_2O/0.1% TFA at a flow rate of 1 mL/min. The reference wild type peptide lacking the azobenzene unit was prepared according to the above-described method except for the coupling step of the azobenzene unit. The desired peptide fraction was lyophilized and characterized by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS): the azobenzene peptide, calcd. 7428.64, obsd. ($[M + H]^+$) 7429.93; the reference peptide, calcd. 7220.43, obsd. ($[M + H]^+$) 7221.69.

Peptide concentration of the azobenzene peptide was determined based on the optical intensities at 320 nm (ε_{320} = 27,780 $M^{-1}cm^{-1}$). Zinc ion (3 equivalents to the apo-peptide) was added into a solution of the apo-peptide in the presence of 1 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP) that prevents oxidation of cysteine residues.

CD spectroscopy. CD spectra were obtained using a Jasco J-720. Measurements were carried out in 10 mM Tris-HCl buffer (pH 7.5) containing 50 mM NaCl and 0.1 mM TCEP in a capped 1-mm path length cell at 4 °C under nitrogen. All spectra represent the average of 8–16 scans. Spectra were baseline-corrected using the Jasco software. CD intensities are expressed as mean residue ellipticities ($dmol•cm^2•mol^{-1}$) calculated by $[\theta] = \theta/lcn$, where θ is the ellipticity observed (mdeg), l is the path length of the cell (cm), c is the peptide concentration (M), and n is the number of peptide bonds in the sequence.

Ultraviolet-visible (UV-vis) absorption spectroscopy. UV-vis absorption spectra were recorded on a Shimadzu UV-2550 at 20 °C in 10 mM Tris–HCl buffer (pH 7.5) containing 50 mM NaCl, 0.1 mM TCEP and $ZnCl_2$ (concentration was described in figure captions.) in a capped 1-cm path length cell.

Photoisomerization. For the trans→cis isomerization, 3UV transilluminator (LMS-20E, UVP) was used at 365 nm. For the cis→trans back isomerization, a halogen lamp (Megalight 100, Hoya-Schott) was used at > 420 nm with a short wave cutoff filter (SCF-50S-42L, Sigma Koki Co., Ltd.). The sample solution was illuminated directly in a capped quartz cuvette at 4 °C.

Gel mobility shift assays. The target DNA containing GC box (G-strand: 5'-TTTA TAT TAA ATA TTA TGG GGC GGG GCC AAT ATA TTA-3', C-strand: 5'-TAA TAT ATT GGC CCC GCC CCA TAA TAT TTA ATA TAAA-3', the GC box is shown in underlined bases.) were

radiolabeled at 5'-tarminus of G-strand by reaction with the T4 polynucleotide kinase and [γ-^{32}P]ATP. The labeled G-strand was annealed with the unlabeled complement C-strand by cooling from 90 °C to 20 °C slowly. Gel mobility shift assays were carried out under the following conditions. Each reaction mixture containing the ^{32}P-labeled target DNA (500 cpm, ~50 pM) and the zinc finger peptide (0–2.5 μM) was incubated in 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 100 μM $ZnCl_2$, 1 mM TCEP, 0.05% Nonidet P-40, 5% glycerol, 40 ng/μL bovine serum albumin (BSA), and 100 ng/μL poly(dI-dC) for 30 min at 4 °C. In the case of photoisomerization, the reaction mixture was further incubated under UV light for 30 min at 4 °C. After incubation, the reaction mixture was analyzed by electrophoresis on a 12% 19:1 acrylamide-bisacrylamide gel in 1 × Tris-borate (TB) buffer at 4 °C under UV or ambient light. The bands were visualized by autoradiography and quantified using Image Gauge version 4.01 software (Fujifilm Corporation). The dissociation constant (*K*d) of the peptide for the target DNA was evaluated by curve-fitting the experimentally obtained data to the equation: $F = [P]/([P] + Kd)$, where F and [P] represent the fraction of the peptide-bound DNA and the total peptide concentration, respectively.

GC box cleavage by the restriction enzyme. The cleavage of ^{32}P-labeled target DNA (500 cpm, ~50 pM) by AciI (400 U/mL) were carried out at 37 ºC for 0–30 min in 20 mM Tris-HCl (pH8) containing 100 mM NaCl, 100 μM $ZnCl_2$, 1 mM TCEP, 0.05% Nonidet P-40, 5% glycerol, 40 ng/μL BSA, 100 ng/μL poly(dI-dC), and 10 mM $MgCl_2$ under UV or ambient light. In the presence of the peptide, the target DNA and the peptide (2.5 μM) were pre-incubated for 30 min at 4 °C before the enzyme reaction. After reaction, the cleavage products were extracted from the reaction mixture, and were analyzed by electrophoresis on a 15% denaturing 19:1 acrylamide-bisacrylamide/7 M urea sequencing gel in 1 × Tris-borate-EDTA (TBE) buffer. The bands were visualized and quantified as described above.

References

(1) E. Kaiser, R. L. Colecscott, C. D. Bossinger, P. I. Cook, *Anal. Biochem.,* 1970, **34,** 595–598.

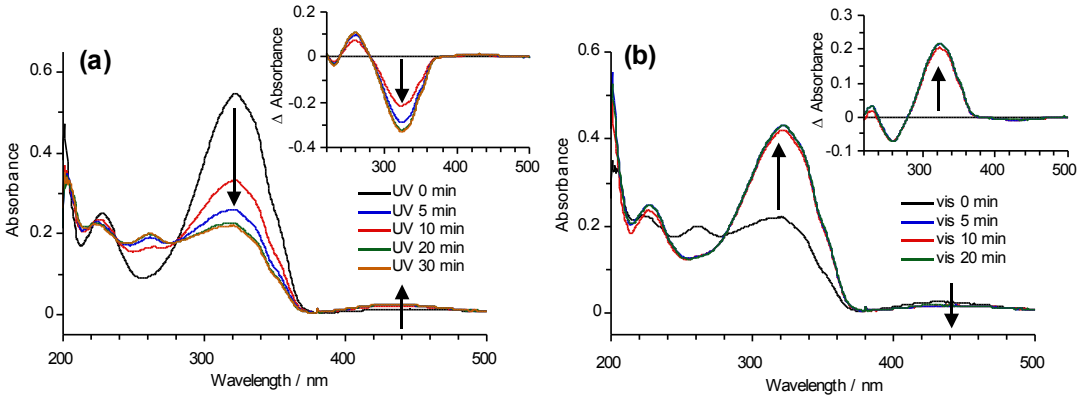

Fig. S1 UV-vis absorption spectral change of 4-phenylazobenzoic chloride (30 μM) under light irradiation in methanol at 20 ºC. (a) UV light (365 nm), (b) visible light (> 420 nm) after UV irradiation. Respective irradiation times are indicated in the figure. Inset: difference spectra.

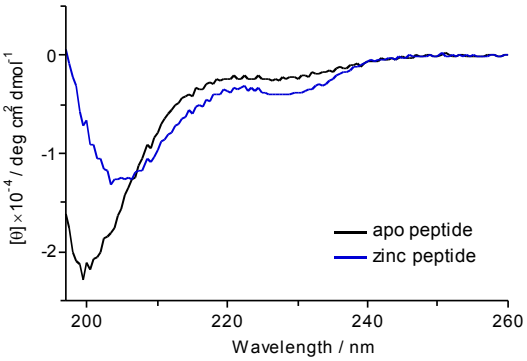

Fig. S2 CD spectra of the unmodified peptide (30 μM) in 10 mM Tris-HCl (pH7.5) containing 50 mM NaCl and 0.1 mM TCEP in both the absence and presence of 3 equivalent $ZnCl_2$ at 4 ºC. Black and blue lines show apo-peptide and zinc peptide, respectively.

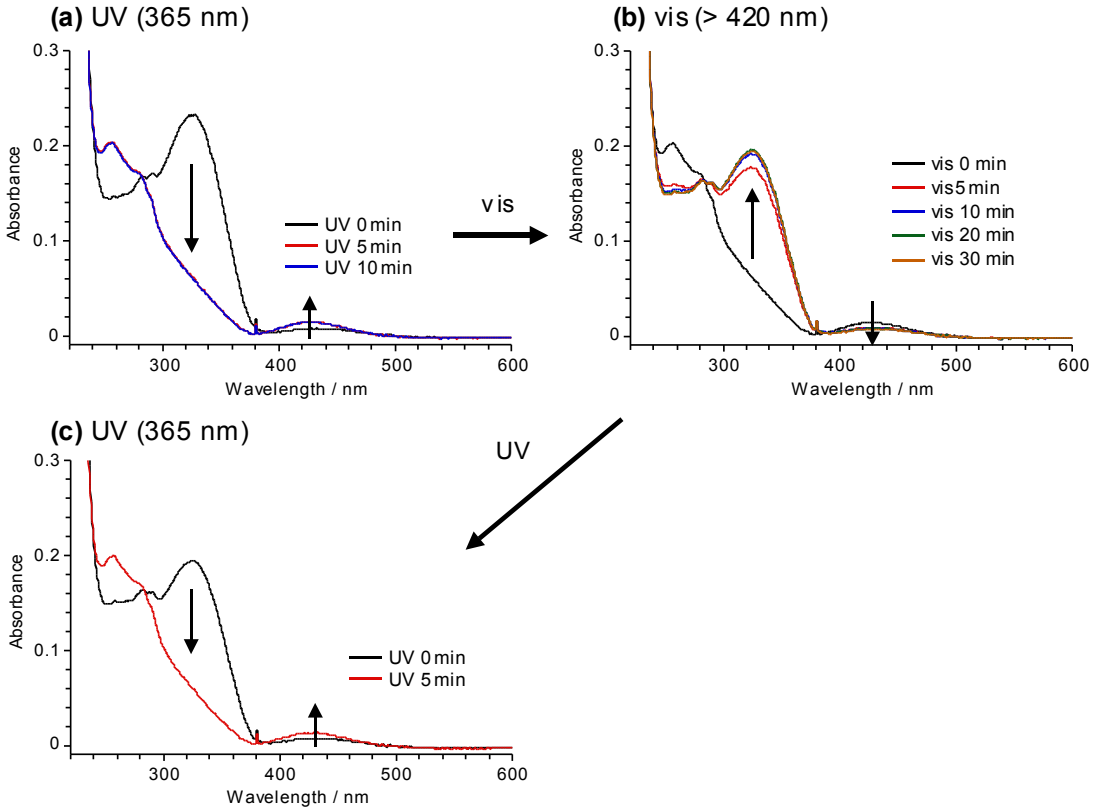

Fig. S3 UV-vis absorption spectral change of Zn-ASP (8.2 μM) under light irradiation in the presence of denaturant Gdn-HCl. Conditions: in 10 mM Tris-HCl (pH7.5) containing 25 μM $ZnCl_2$, 50 mM NaCl, 0.1 mM TCEP, and 1 M Gdn-HCl at 20 ºC. (a) UV light (365 nm), (b) visible light (> 420 nm) after UV irradiation, and (c) UV irradiation after UV and visible irradiation. Respective irradiation times are indicated in the figure.

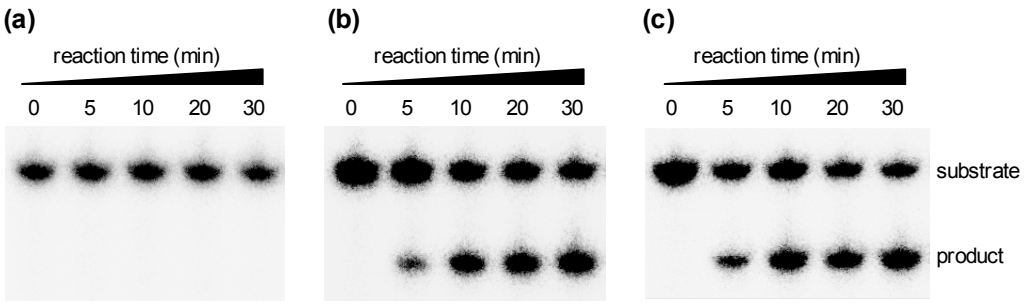

Fig. S4 GC box cleavage by the restriction enzyme *Aci*I in the presence (a: before UV irradiation, b: under UV light during enzyme reaction) or absence (c) of Zn-ASP. The reactions were carried out at 37 ºC in 20 mM Tris-HCl (pH8) containing 100 mM NaCl, 100 μM $ZnCl_2$, 1 mM TCEP, 0.05% Nonidet P-40, 5% glycerol, 40 ng / μL BSA, 100 ng / μL poly(dI-dC), 10 mM $MgCl_2$. The reaction mixtures were analyzed by gel electrophoresis using 7M urea / 15% polyacrylamide gel in 1 × TBE buffer.

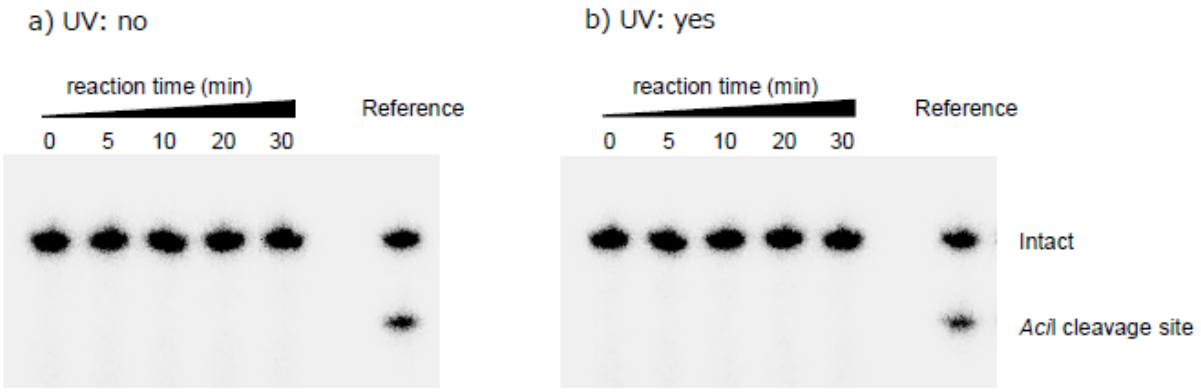

Fig. S5 GC box cleavage by the restriction enzyme *Aci*I in the presence of the unmodified zinc finger peptide (a: before UV irradiation, b: under UV light during enzyme reaction). The reactions were carried out at 37 ºC in 20 mM Tris-HCl (pH8) containing 100 mM NaCl, 100 mM $ZnCl_2$, 1 mM TCEP, 0.05% Nonidet P-40, 5% glycerol, 40 ng/mL BSA, 100 ng/mL poly(dI-dC), 10 mM $MgCl_2$. The reaction mixtures were analyzed by gel electrophoresis using 7M urea / 15% polyacrylamide gel in 1 × TBE buffer.

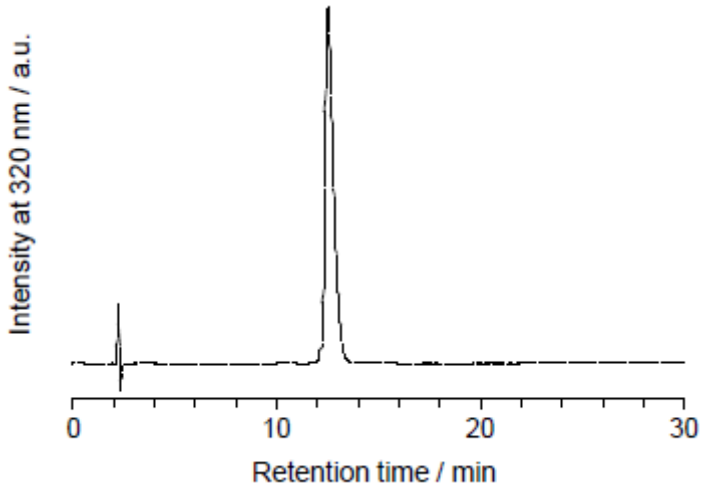

Fig. S6 HPLC profile of apo-ASP. ASP was analyzed by reversed phase HPLC on Chemcobond 5-ODS-H (4.6 mm × 150 mm, Chemco Scientific Co., Ltd.) with a 30-min linear gradient of 25-70% acetonitrile/aqueous 0.1% TFA at a flow rate of 1 mL/min.