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Supplementary Information

Light-Activating Gene Expression by Site-Specifically Caged DNA with a Biotinylated Photolabile Protection Group

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Generation of PCR products

PCRs for site-specific amino-modification of DNA were carried out using PrimeSTAR® HS DNA Polymerase (Takara Bio Inc., Japan) and the following primer sets containing 5'-amino-modified DNA primers: 5'-NH_2-GTCTATATAAGCAGAGC-TGGTTTAGTGAACCGT-3' and 5'-GTAGCGCTAGCGGATCTGACGGTTCACTA-AACC-3' for amino-modification near TATA sequences, and 5'-NH_2-AGGAGGCTTT-TTTGGAGGCCTAGGCTTTTGCAA-3' and 5'-CTGTCTCTTGATCGATCTTTGCA-AAAGCCTAGG-3' for amno-modification at the downstream of poly(A) sequences (all synthetic oligo-DNA from Invitrogen Corp., USA). Amplification of DNA fragments from the pEGFP-N1 (Clontech, USA) was achieved by adding 200 ng of pEGFP-N1, 200 μM each of dNTP, 0.24 μM each of primer, and 1.25 units of PrimeSTAR® HS DNA Polymerase into a PrimeSTAR Buffer of 50μl. These samples were subjected to 30 cycles of denaturation (10 sec, 95 – 98 ºC), annealing (5 – 15 sec, 55 ºC), and extension (5 min, 72 ºC) using PCR Thermal Cycler (Takara Bio Inc., Japan). After the PCR, for fragmentation of template DNA, 20 units of Dpn I (New England Biolabs Inc., USA) was added to the PCR products solutions, and these solutions were incubated for 1 hour at 37 ºC. Subsequently, the PCR products were purified by column chromatography using QIAquick-spin PCR purification kit (Qiagen, USA). The PCR products were analyzed by agarose gel electrophoresis and further purified by cutting out DNA bands from the gel when needed. The purified PCR products were quantified by measuring the absorbance at 260 nm using the NanoDrop ND-1000 micro-spectrophotometer (NanoDrop Technologies, USA).

Synthesis of biotinylated caging agent 1

The starting compound **2** was prepared in three steps from commercially available materials as previously reported.[S1, S2]

Synthesis of 3

To a suspension of **2** (62 mg, 0.15 mmol) in CH_2Cl_2 (9 ml) on an ice bath was added trifluoroacetic acid (1 ml). After stirring for 15 min, the solution was allowed to warm to room temperature and stirred for a further 2 h. The solvent was removed under vacuum and the resulting residue was re-dissolved in anhydrous DMF (3 ml). To the solution were added anhydrous Triethylamine (Et_3N, 200 μl, 1.4 mmol) and Biotin *N*-succinimidyl ester (41 mg, 0.12 mmol). The reaction mixture was stirred at room temperature for 1 h. After evaporation, the crude residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 4/1) to give compound **3** as white

powder (45 mg, 56%).
^{1}H-NMR (*d*-DMSO, 500 MHz): δ 8,01 (br, 1H), 7.92 (s, 1H), 7.26 (s, 1H), 6.39 (s, 1H), 6.34 (br, 1H), 6.34 (s, 1H), 5.65 (br, 1H), 4.72 (s, 2H), 4.28 (t, 1H), 4.19 (t, 2H), 4.10 (d, 1H), 3.46 (m, 2H), 2.78 (m, 1H), 2.56 (d, 2H), 2.10 (t, 2H), 1.59-1.45 (m, 4H), 1.29 (m, 2H).

Synthesis of 1

To a suspension of **3** (100 mg, 0.19 mmol) in CH_2Cl_2 (10 ml) on an ice bath were added Et_3N (120 µl, 0.86 mmol) and 4-nitrophenyl chloroformate (76 mg, 0.38 mmol). After stirring for 15 min, the solution was allowed to warm to room temperature and stirred over night. After evaporation, the crude residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 5/1) to give compound **1** as white powder (10 mg, 8%).
^{1}H-NMR ($CDCl_3$/CD_3OD=5/1 500 MHz): δ 8,34 (d, 2H), 7.98 (s, 1H), 7.78 (s, 1H), 7.48 (d, 2H), 7.41 (br, 1H), 6.99 (br, 2H), 6.50 (s, 1H), 5.48 (s, 2H), 4.50 (t, 1H), 4.29 (d, 1H), 4.20 (t, 2H), 3.72 (t, 2H), 3.15 (m, 1H), 2.73 (d, 2H), 2.27 (t, 2H), 1.72-1.68 (m, 4H), 1.44 (m, 2H).

DNA caging with 1

Compound **1** of 0.2 to 10 µM was agitated with 2 µg of amino-modified PCR products in 40 µl of a sodium carbonate buffer buffer (10 mM, pH 8.2) containing 40 % DMSO for 4 hours at room temperature. In order to remove all excess caging agent **1**, the caged PCR products were purified by column chromatography using QIAquick-spin PCR purification kit and gel filtration using 96 well multiscreen filter plates (Millipore) with Sephadex^{TM} G-50 Fine (Pharmacia).

Evaluation of site-specific caging and photo-cleavage

Site-specific modification of PCR products with biotinylated caging agent **1** was investigated by restriction enzyme fragmentation and electrophoretic mobility shift analysis. 160 ng of site-specifically caged PCR products were double digested with 4 units of *Sma* I (from Takara Bio Inc., Japan) and 4 units of *Sna*B I (from Takara Bio Inc., Japan) in a Tris-acetate buffer (20 mM Tris-acetate, 10 mM magnesium acetate, 50 mM potassium acetate, 1 mM DTT, pH7.9) for 1 h at 37 ºC. Subsequently, 10 ng of Alexa Fluor 488-labeled streptavidin (from from Invitrogen Corp., USA) was added into the digestion mixture for 1 h at room temperature. After addition of 0.25% Orange-G (from Division Chroma, Germany), 1 mM EDTA and 40% sucrose, this

mixture was electrophoresed in 1% agarose gels. The obtained electrophoretic gels were scanned with Typhoon 9400 Scanner (GE Healthcare, USA) using the excitation lasers at 532 nm for EtBr-fluorescent imaging and at 488 nm for Alexa Fluor 488-fluorescent imaging.

Photo-cleavage of site-specifically caged plasmid DNA was investigated in the Tris buffer (20 mM Tris-acetate, 10 mM $MgCl_2$, 0.025% Triton X-100, pH 7.5). The site-specifically caged plasmid DNA solution (16 ng/µl) in 10µl of the Tris buffer was placed in the bottom of a polypropylene microfuge tube (Sorenson^{TM} from NIPPON Genetics Co. Ltd, Japan) without the cap and irradiated from above with UV light through optical fibers and lenses. The UV irradiation of 365 nm (10 nm half bandwidth) was performed using a UV spot light source (100-W Xe lamp, Asahi Spectra Co., Ltd., Japan) and a band pass filter (LX0365, Asahi Spectra Co., Ltd., Japan) at 8 mW/cm^2. The intensity of light was measured with a UV power meter (from Ushio Inc., Japan). The photo-irradiated samples were analyzed by restriction enzyme fragmentation and electrophoresis as described above.

Cell culture and transfection of cells

Human cervical carcinoma derived HeLa cell line (CCL-2) was procured from RIKEN BioResource Center (Japan). HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM, from Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 µg/ml streptomycin (from Sigma-Aldrich, USA) at 37 ºC under 5% CO_2. One day before transfection, 20,000 cells in 500 µl of medium were plated in 24 well plates (from Iwaki, Japan).

Before transfection, 500 ng site-specifically caged DNA was incubated with or without 200 ng streptavidin in a Tris-acetate buffer over night for binding to streptavidin. The DNA-lipofection reagent complex was prepared in reduced-Serum medium (Opti-MEM I from Invitrogen Corp., USA) of 100 µl by mixing 500 ng DNA with 0.5µl PLUS^{TM} Reagent (Invitrogen Corp., USA) and 1.25µl Lipofectamine^{TX} LTX (Invitrogen Corp., USA) in series. These complex solutions were dropped to the well containing cells. Cells were incubated with the DNA-Lipofectamine complex for 2 h.

After transfection, the medium containing the DNA-lipofection reagent complex was replaced with DMEM (10% FBS). The UV treated cells were exposed to UV light from a UV spot light source as described above at 8 mW/cm^2 for the specified time. After culture for 24 hours, 5,000 cells of each sample were analyzed by a flow cytometer equipped with an argon laser (FACS-Calibur, from Becton-Dickinson, USA)). The EGFP-positive cells were determined by a standard gating technique[S3].

Observation with confocal laser scaning microscopy

To confirm the introduction of streptavidin-caged plasmid DNA conjugates into cells, *R*-phycoerythrin-labeled streptavidin (SAPE, from Invitrogen Corp., USA) was employed for visualizing the conjugates. Before transfection, caged or non-caged plasmid DNAs of each 200 ng were incubated with 400 ng SAPE in a Tris-acetate buffer for 4 h. The cells were incubated with SAPE-caged DNA conjugates, Lipofectamine^{TX} LTX and PLUS^{TX} reagent for 4 h as described above. Immediately after replace of medium, the transfected cells were observed by confocal laser scaning microscopy (LSM510, from Carl Zeiss Co., Ltd, Germany) equipped with a He-Ne laser (543 nm).

References

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[S3] W. –C. Tseng, F. R. Haselton, T. D. Giorgio, *J. Biol. Chem.* **1997**, 272, 25641

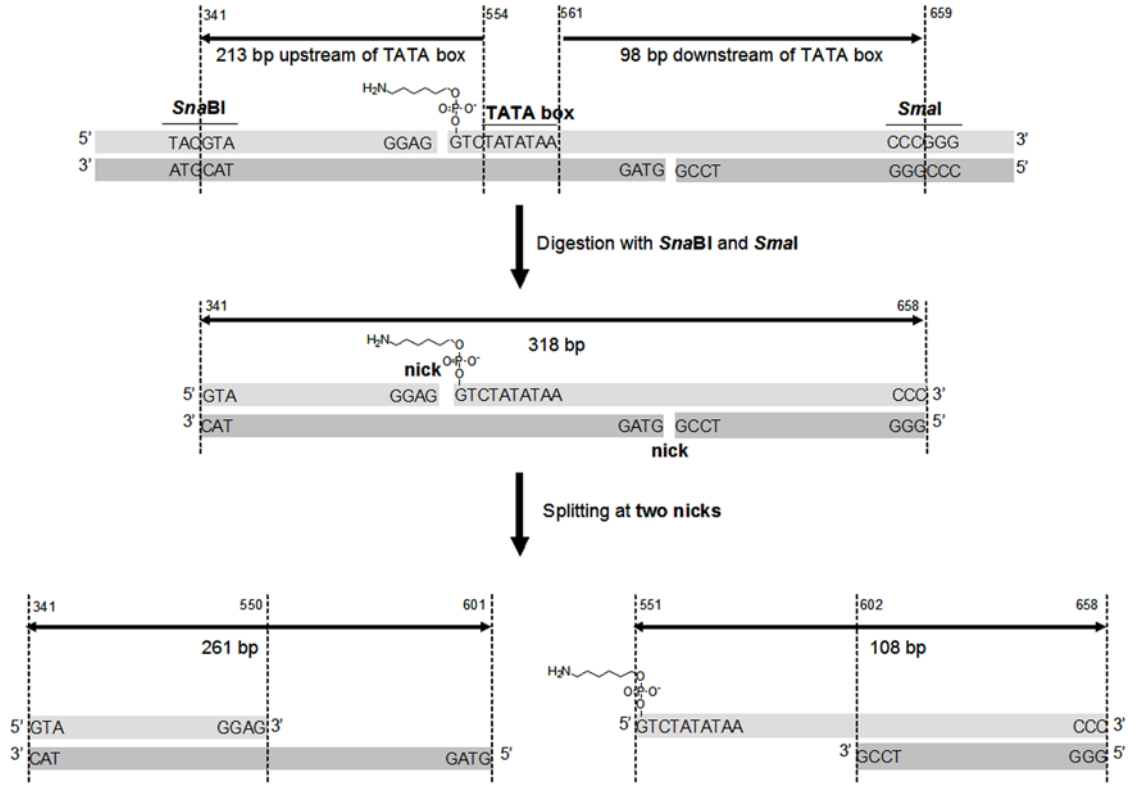

Fig. S1 Schematic illustration of the enzymatic digestion and the splitting of non-caged plasmid during electrophoresis. Site-specifically caged plasmid was digested with restriction enzymes (*SnaB*I and *Sma*I) at two sites: 213 bp upstream (*SnaB*I site) and 98 bp downstream (*Sma*I site) of the TATA box. Then, a DNA fragment of 318 bp was split into two shorter DNA fragments because the DNA duplex between the two staggered nicks probably denatured during electrophoresis.

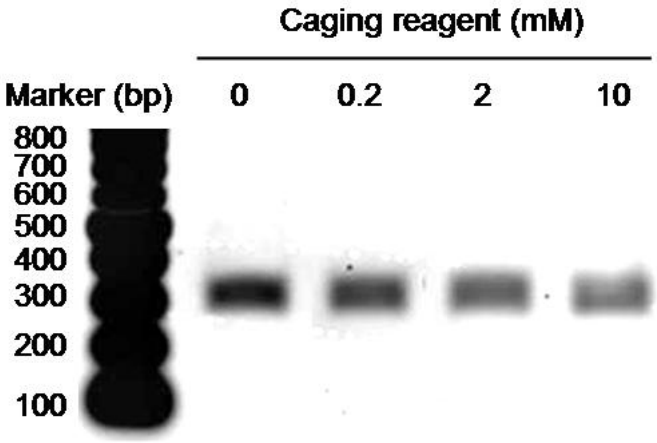

Fig. S2 Electrophoretic mobility shift analysis of the caging reaction of intact pEGFP-N1. The intact pEGFP-N1 was incubated with caging agent **1** at various concentrations of **1** from 0 to 10 mM and digested with two restriction enzymes (*Sma* I and *SnaB* I) as the amino-modified PCR product was done in Fig. 2ab. After electrophoresis, the fluorescent images of gels were obtained by scanning the fluorescence of EtBr.

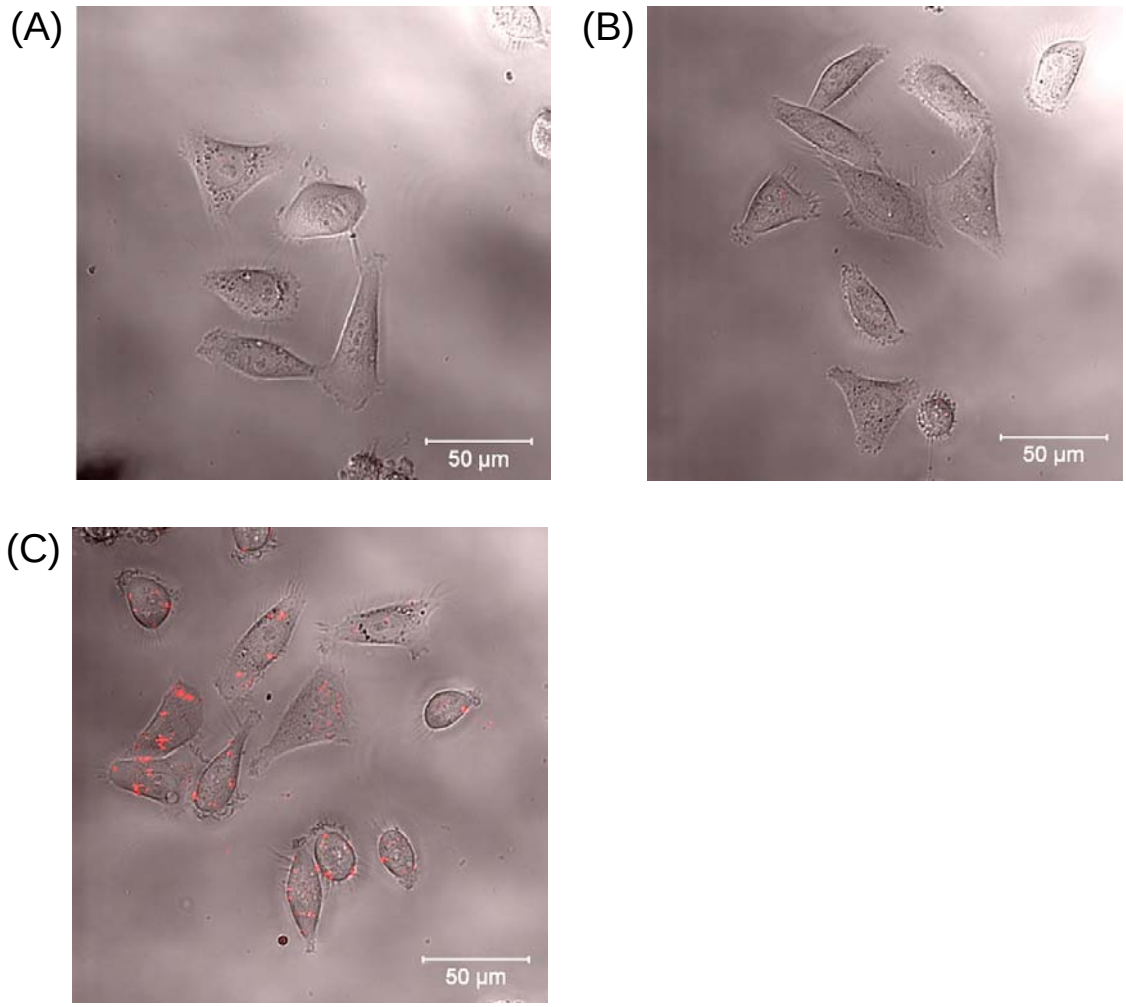

Fig. S3 Introduction of fluorescent-labeled streptavidin-caged DNA conjugates into cells by lipofection. (A) No DNA, (B) non-caged plasmid DNA and (C) caged plasmid DNA were incubated with *R*-phycoerythrin-labeled streptavidin (SAPE) and transferred into HeLa cells by lipofection. After replace of medium, the transfected cells were observed by confocal laser scaning microscopy with a He-Ne laser. The fluorescent images at the emission wavelength above 560 nm and the bright-field images were obtained by excitation at the wavelength of 543 nm. The merge images were prepared by the sum of these two kinds of images and shown. Several red-fluorescent aggregates were observed in **C** whereas no red-fluorescent aggregate was detected in **A** and **B**.