Supporting Information

Photoactivatable fluorescein derivatives with azidomethyl caging groups for tracing oligonucleotides in living human cells

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Synthesis of fluorogenic molecules

Monoazidomethyl fluorescein (MAF) **1** and bisazidomethyl fluorescein (BAF) **3** were synthesized according to the methods we have reported previously.[1]

Synthesis of modified oligonucleotides

To prerare the **ODN 1**, the monoazidomethyl fluorescein NHS ester was reacted with 3′ amino-modified oligonucleotide. 3′ PT-amino-modifier C3 CPG (Glen Research) was used to prepare 3′ amino-modified oligonucleotide. 50 nmol of the 3′ amino-modified oligonucleotide in 50 μl of 80 mM sodium tetraborate (pH 8.5) were shaken for 5 h at room temperature with 0.75 μmol of the NHS-ester derivative of **MAF 1** in 200 μl of dimethylformamide. To prerare the **ODN 3**, the bromoacetyl group of the bis-azidomethyl fluorescein was reacted with the phosphorothioate group on the oligonucleotides. For 3′ phosphorothioate sequences, the 3′-phosphate CPG was sulfurized by the sulfrizing reagent (Glen Research) after the first nucleotide was added. 75 nmol of the 3′-phosphorothioate oligonucleotide in 50 μl of 400 mM triethylammonium bicarbonate buffer were shaken for 5 h at room temperature with 750 nmol of **BAF 3** in 200 μl of dimethylformamide. The both reacted products were collected by ethanol precipitation. Next, the products were purified by reverse-phase HPLC (0–80% acetonitrile/50 mM triethylammonium acetate gradient). The probe structure was confirmed by ESI–TOF mass spectrometry.

ODN 1 5′-TTTGAATATTTGCTA-monoazFlu-3′: calculated mass, $C_{175}H_{210}N_{53}O_{101}P_{15}$ 5133.9; found 5133.2.

ODN 3 5′-TCGGGGCACCCAT-bisazFlu-3′: calculated mass, $C_{152}H_{177}N_{56}O_{84}P_{13}S$ 4564.8; found 4564.7.

Photolysis and spectrum analysis

The photolysis and spectrum analysis were carried out on a UV-VIS spectrometry (V-500; JASCO) and a fluorescence spectrometry (FP-6500; JASCO) using 5 μM of **MAF 1** or **ODN 1**. Solutions for photolysis and spectrum analysis were prepared in 20 mM of Tris-HCl buffer (pH 7.2). The solution was scanned from 350-550 nm for UV-VIS spectroscopic analysis or 500-650 nm with excitation at 490 nm for fluorescence analysis before irradiation and after every 2 minute of irradiation (λirr = 365 nm, 200 mW/cm^2) using SP-7 Spot Cure (USHIO) to monitor the progress of the photocleavage reaction. The reaction product from **ODN 1** after 14 min of

photocleavage was analyzed by reverse-phase HPLC (0-80 % acetonitrile/50 mM triethylammonium acetate gradient) using hydrosphere C18 column (250×4.6 mm).

Cell experiment

Hela cells were cultured in DMEM medium (Wako) containg 10% fetal bovine serum and 1% penicillin/streptomycin. Cells to be imaged were cultured on 35 mm Petri dishes with glass bottoms. 1 μM of **ODN 3** was mixed with transfection reagent (Genescilencer; Funakoshi), and then transfected into cells for 1 hour at 37 °C by standard protocol from manufacture. Before the observation under microscope, cells were washed with PBS twice. Imaging was performed on a Olympus FV1000-D laser scanning confocal microscope with a 60x UPLSAPO 1.35 NA objective and a 413/488 dichroic mirror. Photoactivation was performed with ~0.8 mW of 405 nm laser light through the objective. For simultaneous imaging of photoactivated fluroescein, the 488 nm line of a argon laser was used for fluorescein excitation with a 405/488 dichroic mirror and its emission was collected with 500/600 nm band-pass filter. Fluorescent intensities for all ROIs were analysed by Fluoview 2.0 (Olympus).

Reference

1 K. Furukawa, H. Abe, K. Hibino, Y. Sako, S. Tsuneda and Y. Ito, *Bioconjugate Chem.*, 2009**, 20,** 1026.

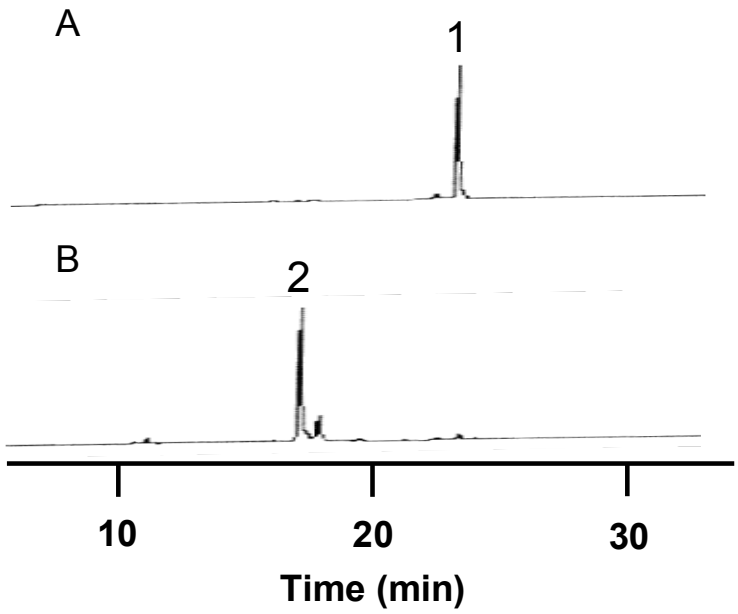


Figure S1 HPLC analysis of ODN 1 before (A) and after 14 min of photodeprotection (B). Peak 1, ODN 1; Peak 2, deprotected form of ODN 1. The reaction products were analyzed by reverse-phase HPLC (0-80 % acetonitrile/50 mM triethylammonium acetate gradient) using hydrosphere C18 column (250×4.6 mm).

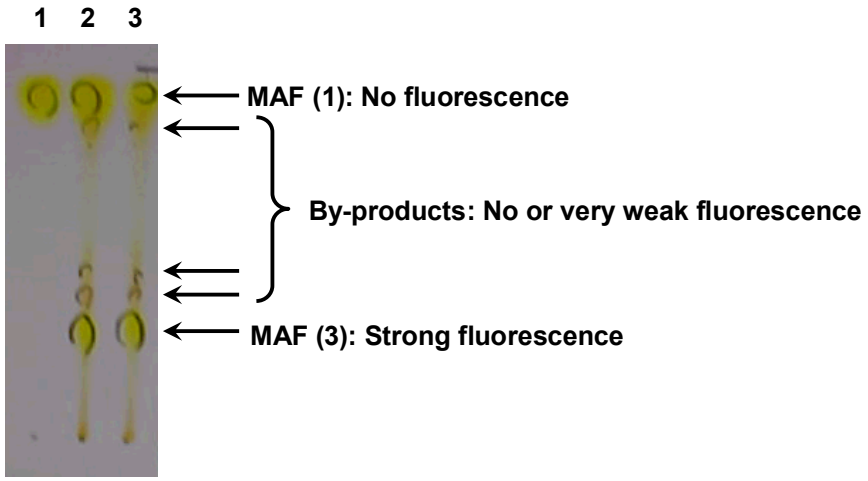


Figure S2 Thin-layer chromatography of MAF (1) after or before UV irradiation for 20 min. The chromatogram was developed in $CHCl_3$/MeOH (10:1). Lane 1: Before UV irradiation, Lane 2: Lane 1+3, Lane 3: After UV irradiation for 20min.

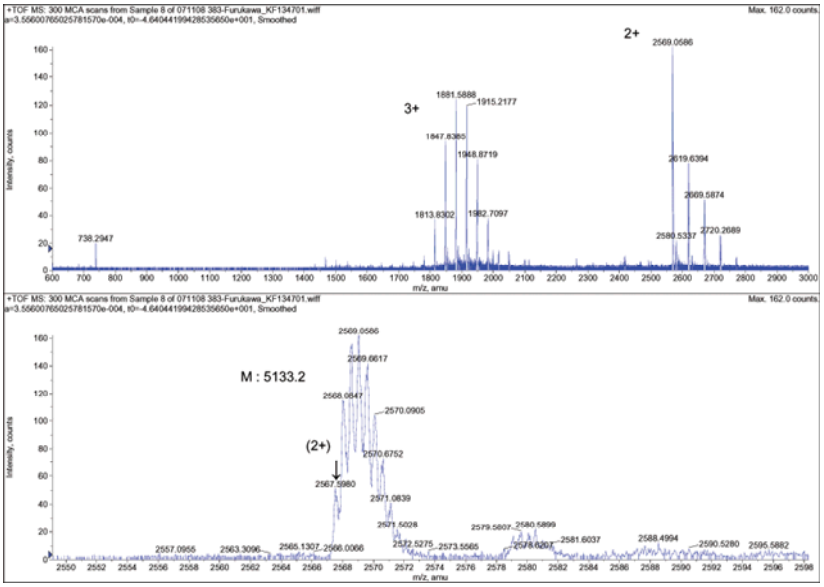
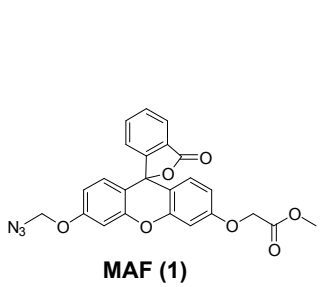


Figure S3 ESI-TOF spectrometry of ODN 1

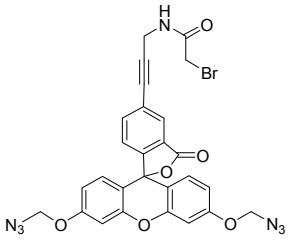
Table S1 Chemical properties and photolysis quantum yields of compound 1-4

	ABS_{max} (nm)	FLU_{max} (nm)	ε_{490}[b]	Φ_{ply}[c]
MAF (1)	n.p.[a]	n.p.[a]	9240	0.221
BAF (2)	n.p.[a]	n.p.[a]	9240	0.664
MAF (3)	455	515	117280	–
BAF (4)	491	516	77800	–

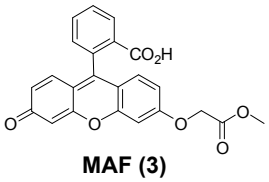
n.p.[a]: No peak at the wavelength longer than 400 nm.
ε_{490}[b] : Extinction coefficients at 490 nm.
Φ_{ply}[c] : Photolysis quantum yields after UV irradiation for 10 min.



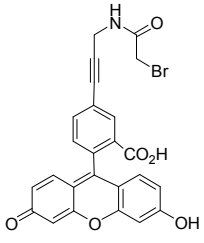
MAF (1)



BAF (2)



MAF (3)



BAF (4)