Supporting Information

An efficient and simple method for site-selective modification of O^6-methyl-2'-deoxyguanosine in DNA

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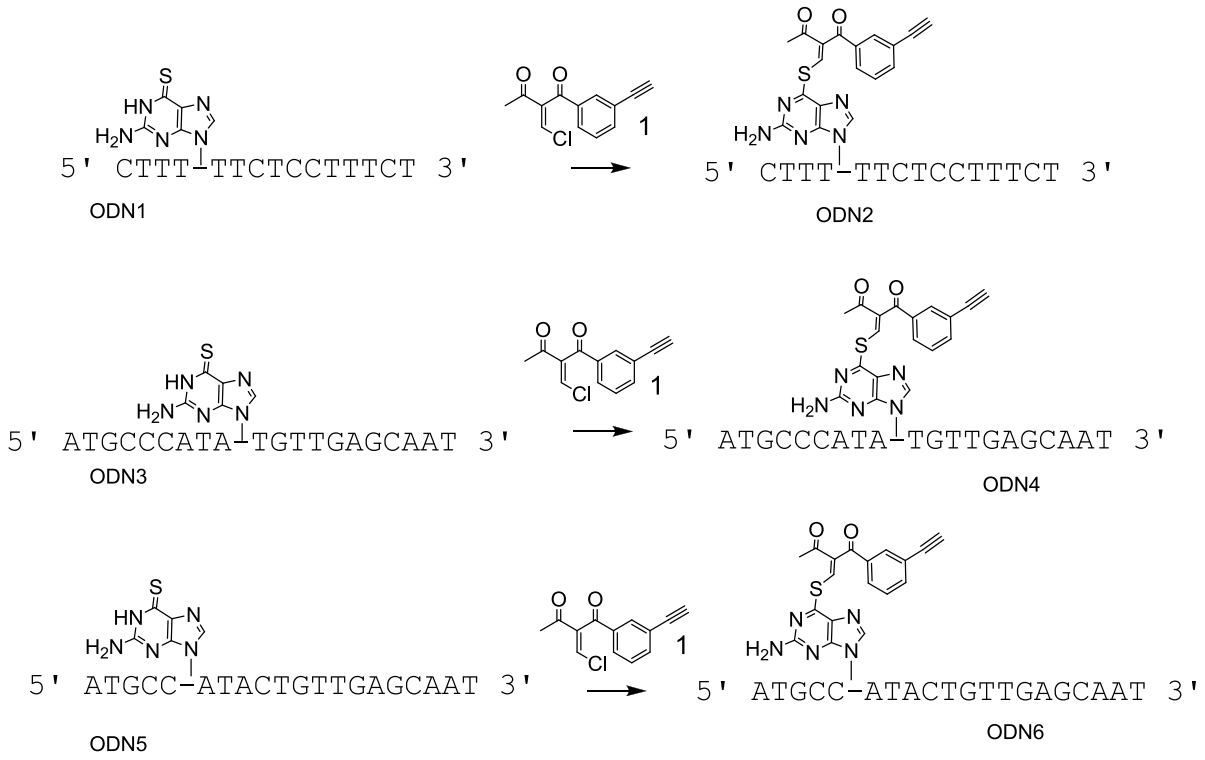


Scheme S1. The structure of the probe ODN.

Synthesis of the functionality-transfer ODN(2, 4 and 5) (FT-ODN)

The synthesis of ODN**2** as a general procedure

A solution of 2 μL ODN**1** (750 μM final concentration, 1.5 nmol) was dissolved in 2.7 μL carbonate buffer (50 mM, pH 10) in a polypropylene microtube. A solution of 0.3 μL **1** (25 mM in CH_3CN, 7.5 nmol) was added to the above solution, and the mixture was kept at room temperature for 10 min. The reaction mixture was checked by HPLC using the following conditions: column: 4.6 × 250 mm (SHISEIDO C18), buffer A: 0.1 M TEAA, buffer, B: CH_3CN, gradient: 10% to 30% /buffer B in 20 min, linear gradient, flow rate: 1.0 ml/min, detection: UV at 254 nm.

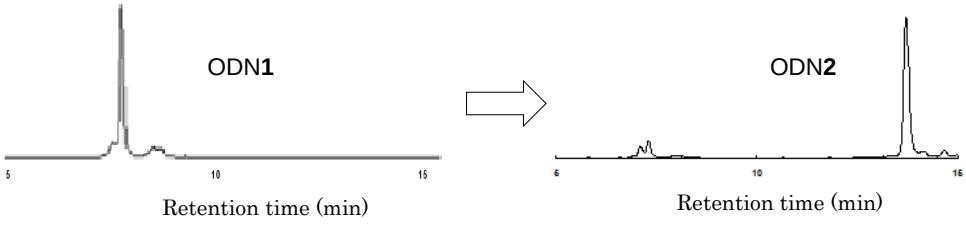
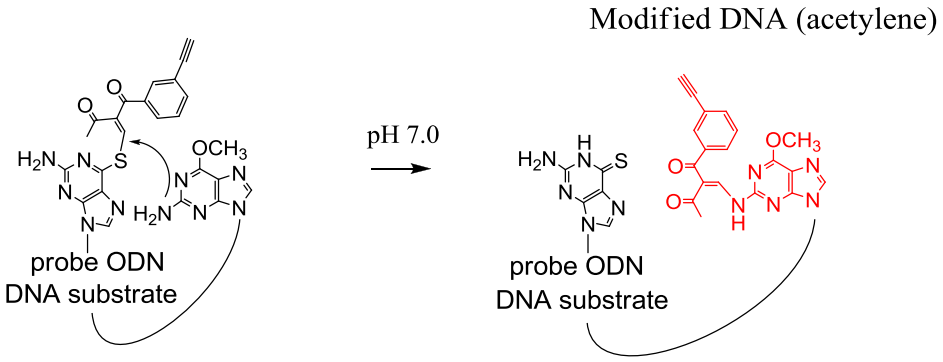


Fig. S1. HPLC change for the synthesis of the ODN probe (**ODN2**).

The functionality transfer reaction

The reaction with ODN**2** and DNA**4** as a general procedure

A solution of 1 μL ODN**2** (200 μM, 200 pmol) was added to 1.0 μL DNA**4** (50 μM, 50 pmol) in MES buffer (1 M NaCl-0.5 M MES, 5 μL, pH 7.0), and diluted with pure water to adjust the volume to 50 μL (ODN**2** at 4 μM final concentration, DNA**4** at 1 μM final concentration, 100 mM NaCl, 50 mM MES buffer final concentration, pH 7.0). The mixture was kept at 25◦C, and the reaction mixture was analyzed by HPLC using the following conditions: column: 4.6 × 250 mm (SHISEIDO C18), buffer A: 0.1 M TEAA buffer, buffer B: CH_3CN, gradient: 10% to 30% buffer B in 20 min, linear gradient, flow rate: 1.0 mL/min, detection: UV at 254 nm. The HPLC profiles are summarized in Fig. 1(A), and the time course is shown in Fig. 1(B).



5' AGAAAGGAGAA**Y**AAAG 6'

DNA**4**: Y=O^6-Me-dG

DNA**5**: Y=dG

5' ATTGTCAACA**–X–**TAT**–Y–**GGCAT 3'

DNA**6**: X=O^6-Me-dG, Y=dG

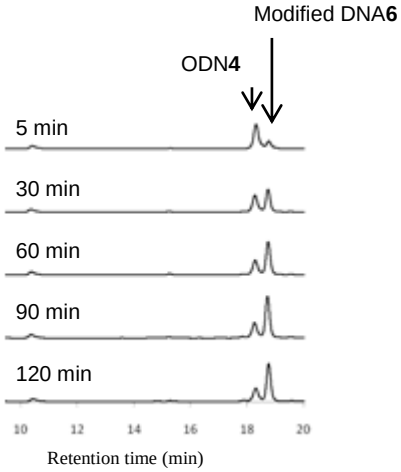
DNA**7**: X=dG, Y=O^6-Me-dG

DNA**8**: X=dG, Y=dG

Fig. S2. The DNA substrates used in this study.

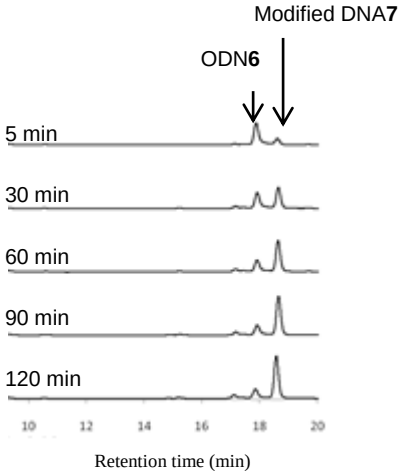
(A) ODN**4** reaction with DNA**6**

ODN**4** decreased and the modified DNA**6** increased.



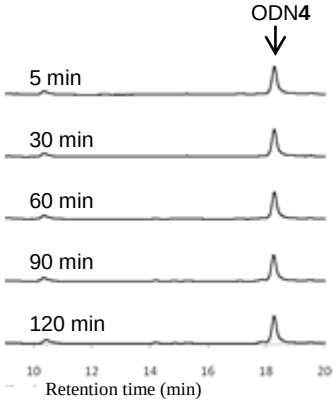
(C) ODN**6** reaction with DNA**7**

ODN**6** decreased and the modified DNA**7** increased.



(B) ODN**4** reaction with DNA**8**

ODN**4** did not change, nor the modified DNA**8** formed.



(D) ODN**6** reaction with DNA**8**

ODN**6** did not change, nor the modified DNA**8** formed.

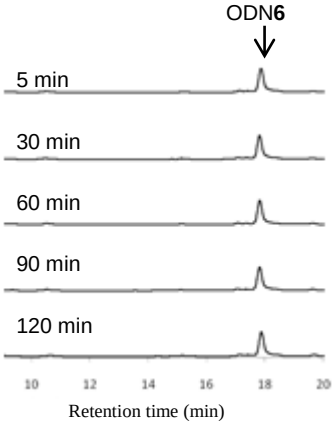


Fig. S3. HPLC change for the functionality transfer reaction. (A) ODN**4** forming the modified DNA**6**, (B) ODN**4** nonreactive to DNA**8**, (C) ODN**6** forming the modified DNA, (D) ODN**6** nonreactive to DNA8. The reaction conditions are described in the general procedure. Column: SHISEIDO CAP CELL PAK C_{18}, buffer A: 0.1 M TEAA, buffer B: CH_3CN, gradient: 10% to 20% buffer B in 20 min linear gradient, flow rate: 1.0 ml/min, column oven at 50^{o}C, detection: UV at 254 nm.

Table S1. MALDI-TOF/MS data of the oligonucleotides.

DNA or RNA	calcd. ([M-H]$^-$)	found
ODN**1**	4767.8	4767.1
ODN**2**	4962.8	4963.0
ODN**3**	6457.0	6457.4
ODN**4**	6653.1	6653.2
ODN**5**	6457.0	6457.3
ODN**6**	6653.1	6653.3
DNA**4**	5056.0	5056.4
Modified DNA**4**(acetylene)	5252.6	5253.0
FAM-DNA**4**	5711.1	5710.1
DNA**5**	5041.9	5041.7
Modified DNA**5**(acetylene)	5238.0	5237.4
Biotin- DNA**5**	5709.2	5709.6
DNA**6**	6455.1	6455.8
Modified DNA**6** (acetylene)	6651.1	6651.7
FAM-DNA**6**	7109.2	7109.8
DNA**7**	6455.1	6455.3
Modified DNA**7**(acetylene)	6651.1	6651.9
FAM-DNA**7**	7109.2	7109.1

Click reaction with FAM-N_3 in the presence of $CuSO_4$

The reaction of the modified DNA**4** as a general procedure

A solution of $CuSO_4$ (0.34 μL, 25 mM in H_2O, 0.15 mM at final concentration) was added into the mixture containing the following solution: a solution of modified DNA**4** (28 μL, 425 pmol after the transfer reaction, 10 μM final concentration), a solution of sodium ascorbate (0.34 μL, 50 mM in H_2O, 0.3 mM final concentration),and TBTA (tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine) (0.34 μL, 50 mM in DMSO, 0.6 mM final concentration) and FAM-N_3 (Fig. 5(B), 4.25 μL, 10 mM in DMSO, 0.75 mM final concentration) and 7.7μL DMSO (DMSO-H_2O, 30% final volume) in a polypropylene microtube. The reaction was monitored by HPLC using the following conditions: column: 4.6 × 250 mm (SHISEIDO C18), buffer A: 0.1 M TEAA, buffer B: CH_3CN, gradient: 10% to 30% buffer B in 20 min, 30% to 100% buffer B in 25 min, flow rate: 1.0 mL/min, detection: UV at 254 nm. The HPLC change is shown in Fig. 5(A).

The protocol for fluorescent detection of *O*^{6}-Me-dG in 80 mer DNA

Step 1: The mixture of ODN**2** (2.8 μL of 150 μM solution, 15 μM at final concentration), 80 mer DNA sample (DNA**1,** 0.5, 1.0, 2.5, 5.0 or 7.5 μL of 1.0 pmol/μL solution, or 0.2, 0.5, 1.0, 1.5 or 2.0 μL of 50 pmol/ μL solution), the MES buffer (2.8 μL of 0.5 M solution, 50 mM at final concentration, pH 7.0) and NaCl solution (2.8 μL of 1M solution, 100 mM at final concentration) was diluted with MilliQ water to 28 μL final volume, and kept at room temperature for 1 hour.

Step 2: Into the above mixture were added FAM-azide in DMSO (4.25 μL of 10 mM solution, 42.5 nmol), sodium ascorbate in H_2O (0.34 μL of 50 mM solution, 17 nmol), TBTA in DMSO (0.34 μL of 50 mM solution), $CuSO_4$ in H_2O (0.34 μL of 25 mM solution, 8.5 nmol) and 7.7 μL of DMSO, and the mixture was kept at room temperature for 30 min.

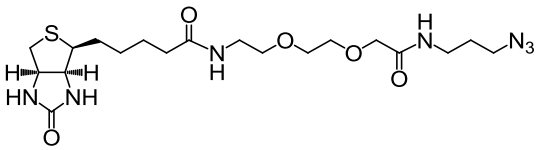
Step 3: The reaction mixture was diluted with 20 μL of the loading buffer (90% formamide, 20 mM EDTA), and treated with 2.2 μL of 28% aqueous ammonia for 10 min, then acidified with 45 μL of aqueous 10 % citric acid.

Step 4: The reaction mixture was diluted with saturated aqueous NaCl solution (100 μL), extracted with methyl isobutyl ketone (300 μL, 12 times) to remove small molecules.

Step 5: The aqueous layer was filtered through a membrane filter (Amicon Ultra, 0.5 mL, 10K) and washed with MilliQ (0.5 mLx30 min, 5 times) by centrifugation (10,000 g). The modified DNA**1** on the filter was dissolved in 5 mM MES buffer (1.5 mL), and the whole solution was subjected to the fluorescent measurement with excitation at 494 nm at 25 ^{o}C. The fluorescent spectra and the dose-dependency thus obtained are shown in Fig. 6.

The protocol for the biotin labeling and following chemiluminescent detection of *O*^{6}-Me-dG in DNA

The labeling protocol from step 1 to 5 was the same as above except that the biotin-azide shown below was used instead of FAM-azide.



Biotin-azide

1 μL of the solution of the labeled DNA was applied on the membrane (Hybond-N+), and heated in the oven for 2 hours at 80^{o}C. The membrane was blocked for 1 hour with the blocking solution (5% skim milk in TBST), and treated with streptavidin-alkaline phosphatase (20 μL in 5 mL of Blocking one) for 2 hours, then successively washed

with the blocking solution for 10 min and TBST for 10 min twice. The membrane was immersed in the CDP-Star substrate solution (0.25 mM), and chemiluminescence was measured by LAS4000. The image and the quantified date are shown in Fig. S4. TBST: Tris-HCl, 50 mM; NaCl, 150 mM; Tween 20, 0.05 %. Blocking one and streptavidin alkaline phosphatase were purchased from Nacalai and R&D Systems, respectively.

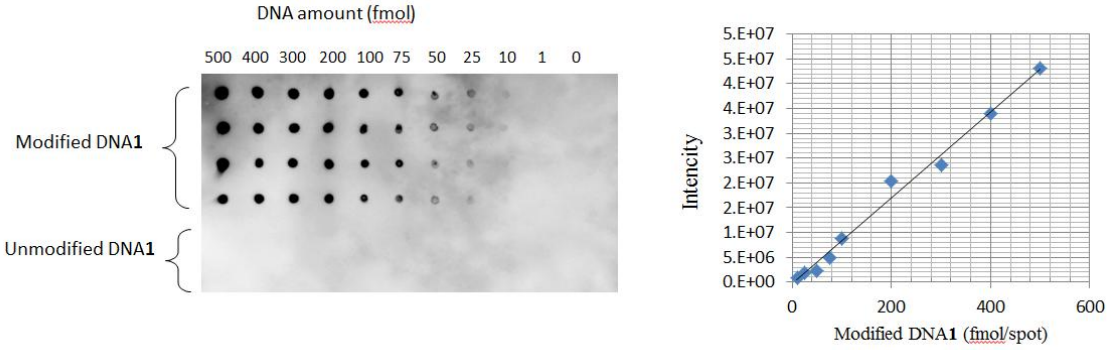


Fig. S4. Detection of biotin-labelled DNA1 by chemiluminescence.