Supplementary Material (ESI) for Chemical Communications

An enzymatic method for site-specific labeling of recombinant proteins with oligonucleotides

Supplementary Information

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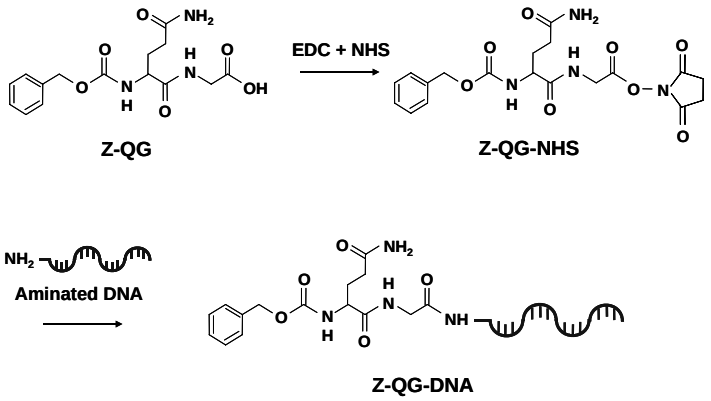
Experimental details

1. Preparation of Z-QG-DNA

The preparation scheme of Z-QG-DNA is represented in Scheme S1. *N*-Carbobenzyloxy glutaminyl glycine (Z-QG, 0.5 mmol) and *N*-hydroxysuccinimide (NHS, 0.5 mmol) were dissolved in dimethyl sulfoxide (DMSO, 1 ml). Synthesis of NHS-activated Z-QG (Z-QG-NHS) was initiated by the addition 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC, 0.6 mmol) dissolved in DMSO (1 ml) and the reaction mixture was stirred overnight at 25°C. Two hundred microliters of the reaction mixture containing NHS-activated Z-QG (Z-QG-NHS) was directly added to the solution of 100 nmol of the 24-mer 5'-aminated DNA (5'-NH_2-$(CH_2)_6$-AGC GGA TAA CAA TTT CAC ACA GGA-3') dissolved in 100 mM borate buffer, pH 9 (1 ml), and the solution was stirred overnight at 25°C to promote the coupling of Z-QG-NHS with the aminated-DNA. The reaction products were subjected to gel-filtration chromatography using an NAP-10 column (Amersham Biosciences) with deionized water as eluent for the purification of Z-QG-DNA.



Scheme S1 Preparation of Z-QG-DNA conjugate

The synthesis of Z-QG-DNA was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF-MS) (Voyager DE-RP, PerSeptive Biosystems) using 3-hydroxypicolinic acid as a matrix (Fig. S1). The conversion rate of an aminated DNA to Z-QG-DNA was estimated by HPLC analysis with an Inertsil® ODS-3 column (GL sciences). The mobile phase consisted of A: 0.1 M TEAA (0.1 M triethanol amine at pH 7.0 adjusted by acetic acid) and B: acetonitrile, and the following gradient was used: 0-15 min (10-25% B); 10-20 (25-70% B); 20-30 min (70-90% B); 30-40 min (90-10% B). The retention times of aminated-DNA and Z-QG-DNA were 11.6 min and 15.0 min, respectively, and under the present reaction conditions, about 98 % of the aminated DNA was labeled with Z-QG.

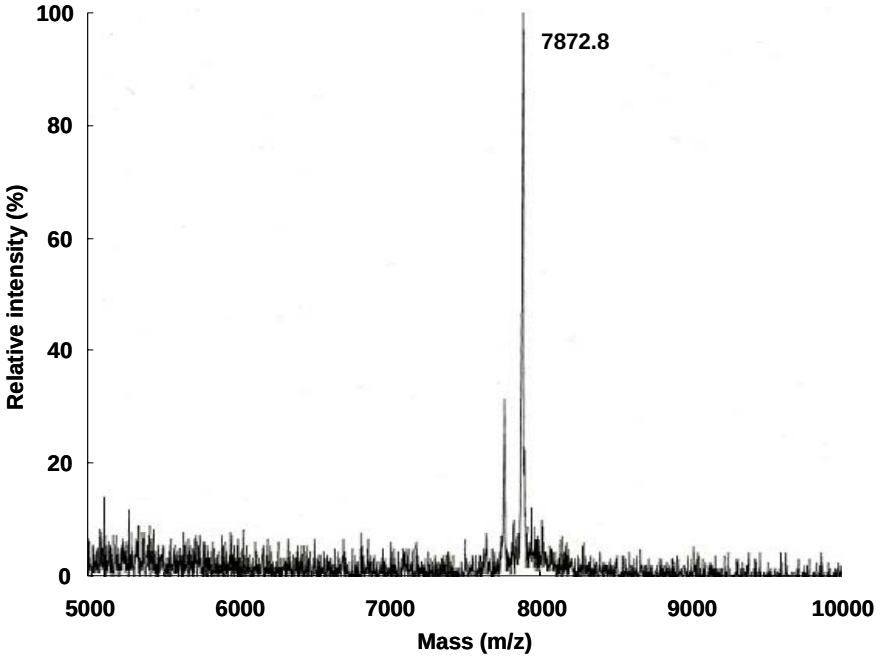


Figure S1 Mass spectrometry of Z-Q-DNA (M_{theor} of Z-QG-DNA is 7873.3)

2. Preparation of recombinant proteins tagged with a specific peptide tag for MTG

A specific peptide consisting of six amino acids (MKHKGS = K6-tag) was genetically attached to the *N*-termini of AP and EGFP, and to the *C*-terminus of AP. The resultant *N*-terminal K6-tagged AP and EGFP and the *C*-terminal K6-tagged AP were abbreviated as NK6-AP, NK6-EGFP and CK6-AP respectively. NK6-AP and CK6-AP were prepared as previously reported.[1,2] The preparation of NK6-EGFP was as follows. A DNA fragment encoding NK6-EGFP was amplified by the polymerase chain reaction (PCR) using pET32-b562-EGFP[3] as the template DNA, which was kindly provided by

Prof. Teruyuki Nagamune of the University of Tokyo. The primer nucleotide sequences used for PCR were 5'-GGA ATT CCA TAT GAA ACA TAA AGG ATC CAT GGT GAG CAA GGG CGA GG-3' and 5'-CCG GAA TTC CGG TTA TCA GTG GTG GTG GTG GTG GTG GTA CAG CTC GTC CAT GCC GAG-3'. The resultant gene fragment encoding NK6-EGFP with *Nde*I and *EcoR*I sites was cloned into a bacterial expression plasmid vector, pET22b(+) (Novagen), by digestion with the restriction enzymes (pET22b-NK6-EGFP). NK6-EGFP was expressed in *E. coli* strain BL21. The fusion proteins obtained were purified by the hexahistidine-tag (His-tag) attached to the *C*-terminus of NK6-EGFP. Briefly, cell-lysate was first loaded onto a Ni-NTA column (column volume: 5 ml, Amersham Biosciences), which was equilibrated with 20 mM Tris-HCl (pH 7.4) supplemented with 500 mM NaCl and 35 mM imidazole. The column was then washed with 50 ml of the equilibrated buffer. The recombinant protein was eluted with 20 mM Tris-HCl (pH 7.4) supplemented with 500 mM NaCl and 500 mM imidazole. Finally, the aqueous solution of NK6-EGFP was desalted using a PD-10 column (Amersham Biosciences) with deionized water as eluent.

3. MTG-mediated conjugation of *N*,*N*-dimethylated casein with an aminated-DNA

N,*N*-Dimethylated casein was purchased from Sigma-Aldrich and employed without further purification. *N*,*N*-Dimethylated casein (4 μM) and an aminated DNA (50 μM) were dissolved in 50 mM Tris-HCl buffer at pH 7. The conjugation reaction was initiated by the addition of MTG (1.1 U/ml) at 25°C. After incubation for 3 h, the reaction products were analyzed by sodium dodecyl sulfate-poly-acrylamde gel electrophoresis (SDS-PAGE). Proteins were stained with Coomassie Brilliant Blue (CBB), and the protein band intensities were quantified by image analysis using the software CS analyzer (ATTO, Tokyo, Japan) on a personal computer. Figure S2 shows the SDS-PAGE of the reaction products. The band of *N*,*N*-dimethylated casein was not shifted to a higher molecular weight, and no significant change was observed by image analysis regarding the absence or the presence of MTG (lanes 2 and 3 in Fig. S2).

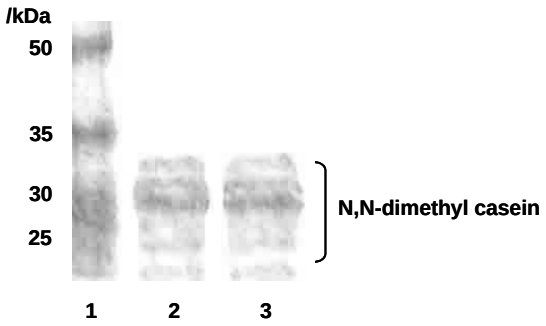


Figure S2. SDS-PAGE analysis of MTG-mediated conjugation of *N*,*N*-dimethylated casein with an aminated DNA (lane 1, molecular weight markers; lane 2, *N*,*N*-dimethylated casein; lane 3, *N*,*N*-dimethylated casein and aminated-DNA treated with MTG). The protein bands observed around 20 – 30kDa were of *N*,*N*-dimethylated casein.

4. MTG-mediated conjugation of recombinant proteins with Z-QG-DNA

Each NK6-tagged recombinant protein (6 μM) and Z-QG-DNA (6, 25, 50 μM) were dissolved in 50 mM Tris-HCl buffer at pH 7. The conjugation reaction was initiated by the addition of MTG (1.1 U/ml) at 25°C. After incubation for 3 h, the reaction products were analyzed by SDS-PAGE. Figure S3 shows the SDS-PAGE analysis of the conjugation reactions of NK6-EGFP and Z-QG-DNA. The band of NK6-EGFP was shifted to a higher molecular weight after the reaction with Z-QG-DNA by MTG, as observed in Figure 1 in the manuscript.

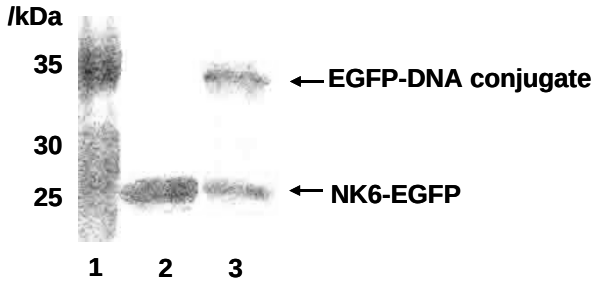


Figure S3. SDS-PAGE analysis of MTG-mediated conjugation of NK6-EGFP to Z-QG-DNA (lane 1, molecular weight markers; lane 2, NK6-EGFP; lane 3, NK6-EGFP and Z-QG-DNA treated with MTG).

In the case of MTG-mediated conjugation in the presence of excess amount of an acyl acceptor, Z-QG-DNA (1.6 μM) and NK6-AP (1.6, 3.2, 8, 16 μM) were dissolved in 50 mM Tris-HCl buffer at pH 7. The conjugation reaction was initiated by the addition of MTG (1.1 U/ml) at 25°C. After incubation for 12 h, the reaction products were analyzed by HPLC. The analytical condition of HPLC was same as described in the preparation of Z-QG-DNA.

5. Effect of MTG-mediated conjugation of Z-QG-DNA on the function of NK6- and CK6-AP, and NK6-EGFP

The catalytic activities of AP were measured with *p*-nitrophenylphosphate (p-NPP) as a substrate. In 1 ml of 1 M Tris-HCl buffer (pH 8), the hydrolysis of p-NPP (1 mM) was initiated by the addition of each recombinant AP at 25°C. The initial activity was determined by the increase in the absorbance at 410 nm derived from *p*-nitrophenol on a UV/Vis spectrophotometer V-560 (Jasco). The AP activities of NK6- and CK6-AP after the conjugation reaction with Z-QG-DNA by MTG compared with the free enzymes were about 99 and 96 %, respectively. The fluorescence spectrum of EGFP was measured by Luminescence spectrometer LS-55 (Perkin Elmer). The fluorescent intensity (excitation: 489 nm, emission: 509 nm) of NK6-EGFP after the conjugation with Z-QG-DNA by MTG was comparable to that of NK6-EGFP before conjugation (≥99 %).

6. DNA-directed immobilization (DDI) of protein-DNA conjugates

All protein-DNA conjugates were separated from free Z-QG-DNA by a Ni-NTA column using the His-tag of the recombinant proteins, prior to DNA-directed immobilization experiments. First, the reaction mixture was loaded onto the Ni-NTA column (column volume: 1 ml, Amersham Biosciences), which was equilibrated with 20 mM Tris-HCl (pH 7.4) supplemented with 500 mM NaCl and 35 mM imidazole. Next, the column was washed by 10 ml of the equilibrated buffer. Protein-DNA conjugates were eluted with 20 mM Tris-HCl (pH 7.4) supplemented with 500 mM NaCl and 500 mM imidazole. The buffer components of the eluted aqueous solution containing protein-DNA conjugates were exchanged by PD-10 column with TBS (Tris buffered saline: 25 mM Tris, 137 mM sodium chloride, 2.68 mM potassium chloride, pH 7.4) as eluent.

DDI was carried out with Nunc streptavidin-coated microplates (Nalge Nunc) with the aid of a microplate washer (MODEL 1575, Bio-Rad) and a fluorescence imager (Molecular imager® FxPro, Bio-Rad). The washing buffer solution (TBST) employed was prepared from TBS by adding 0.05% (v/v) polyoxyethylene sorbitan monolaurate (Tween 20). Prior to use, the microplate was pre-washed three times with TBST, and the 24-mer 5'-biotinylated cDNA (5'-biotin-$(CH_2)_6$-TCC TGT GTG AAA TTG TTA TCC GCT-3', 500nM) was added and incubated at room temperature for 1 h (100 μl/well). After washing the microplate three times with TBST, NK6- or CK6-AP-DNA conjugate (0.8 nM) or NK6-EGFP-DNA conjugate (60 nM) was added to the wells displaying cDNA, incubated at room temperature for 1 h (100 μl/well) and washed three times again with TBST. Finally, in the case of NK6- and CK6-AP, 150 μl of ECF® substrate solution (Amersham Biosciences) diluted ten-fold by deionized water was added to each well, and left for 1 h.

When employing the NK6-AP–Z-QG-DNA conjugate obtained under the reaction conditions of Figure 2 ([NK6-AP]/[Z-QG-DNA] = 5), the reaction products were directly applied to the wells displaying the cDNA (lane 3 in Fig. S4). The analytical condition of DDI was same as mentioned above. A negative control experiment of DDI with an avidin-coated microplate displaying a non-complementary DNA was also conducted (lane 2 in Fig. S4).

Figure S4 DNA-directed immobilization of NK6-AP–Z-QG-DNA conjugate without purification after the MTG-mediated conjugation. The conjugate was immobilized on the cDNA-display microplates (lane 3). As controls, conjugate was applied to an intact avidin-coat microplate (lane 1) or an non-complementary DNA displayed microplate (lane 2) (The sequence of non-complementary DNA: 5'-biotin-$(CH_2)_6$-ACC CTT CCT C-3').

In the case of NK6-EGFP, 150 μl of TBS was added to each well, and the fluorescence intensities of each well were measured using a fluorescence imager (excitation: 488 nm, band pass filter: 530 nm). Figure S5 shows the fluorescence image of the avidin-coated 96-well microplate displaying the biotinylated cDNA after hybridization of NK6-EGFP-DNA conjugate. The substantial fluorescent signal was detected only in the combination of a protein-DNA conjugate and a cDNA-display microplate (lane 3).

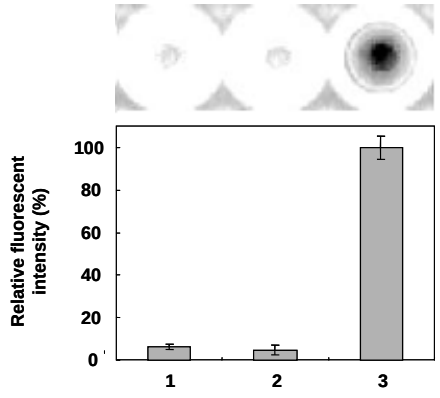


Figure S5. DNA-directed immobilization of NK6-EGFP-DNA conjugate on the cDNA-display microplate (lane 3). As controls, intact NK6-EGFP was immobilized on cDNA-display microplate (lane 1), and NK6-EGFP-DNA conjugate was immobilized on an intact avidin-coated plate (lane 2).

References

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2. Tominaga, J.; Kamiya, N.; Doi, S.; Ichinose, H.; Maruyama, T.; Goto, M. *Biomacromolecules*. **2005**, *6*, 2299-2304.
3. Takeda, S.; Kamiya, N.; Arai, R.; Nagamune, T. *Biochem*. *Biophys*. *Res*. *Commun*. **2001**, *289*, 299-304.