Electronic Supplementary Information

Blinking triggered by the change in the solvent accessibility of the fluorescent molecule

Kiyohiko Kawai,[a,b] Takeshi Koshimo,[a] Atsushi Maruyama,[c] and Tetsuro Majima[a]

[a] *The Institute of Scientific and Industrial Research (SANKEN), Osaka University, Mihogaoka 8-1, Ibaraki, Osaka 567-0047, Japan.*

[b] *PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan.*

[c] *Department of Biomolecular Engineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta 4259, Midori-ku, Yokohama 226-8501, Japan.*

Experimental Methods

DNA Synthesis. Aminolinker-modified DNA were purchased from Gene Design Inc., which were synthesized according to the reported procedure.[1,2] MB and R6G were attached to DNA via the reaction of the amino-linker modified DNA using their succinimidyl derivatives according to the standard procedure, where the incorporation yield was in the range of 20-40%.[1,2] All of the DNA studied here were purified by reverse phase HPLC, lyophilized, and characterized by MALDI-TOFF mass spectra and their concentrations were determined by complete digestion with nuclease P1 and AP to 2'-deoxyribonucleosides, and by UV-absorption method.

Laser flash photolysis. The nanosecond transient absorption measurements were performed using the laser flash photolysis technique.[3-7] Briefly, the second-harmonic oscillation (532 nm, FWHM of 4 ns, 10 mJ / pulse) from a Q-switched Nd:YAG laser (Continuum, Surelight) was used for the excitation light which was expanded to a 1-cm diameter. The light from a xenon flash lamp (Osram, XBO-450) was focused into the sample solution for the transient absorption measurement. Time profiles of the transient absorption in the UV-visible region were measured with a monochromator (Nikon, G250) equipped with a photomultiplier (Hamamatsu Photonics, R928) and digital oscilloscope (Tektronics, TDS-580D). The time profiles were obtained from the average of 16 laser shots and repeated for three times.

FCS. The FCS measurements were carried out using the MF20 (Olympus)[1,2,8] in an aqueous solution. He-Ne laser (543 nm, 50 μW) was used as the excitation source. A twofold higher concentration of complementary strand compared to R6G-modifed strand was used in the case of duplex. All experiments were performed with 10 s of data acquisition time per measurement, and repeated 4-12 times per sample.

Absorption and fluorescence spectra

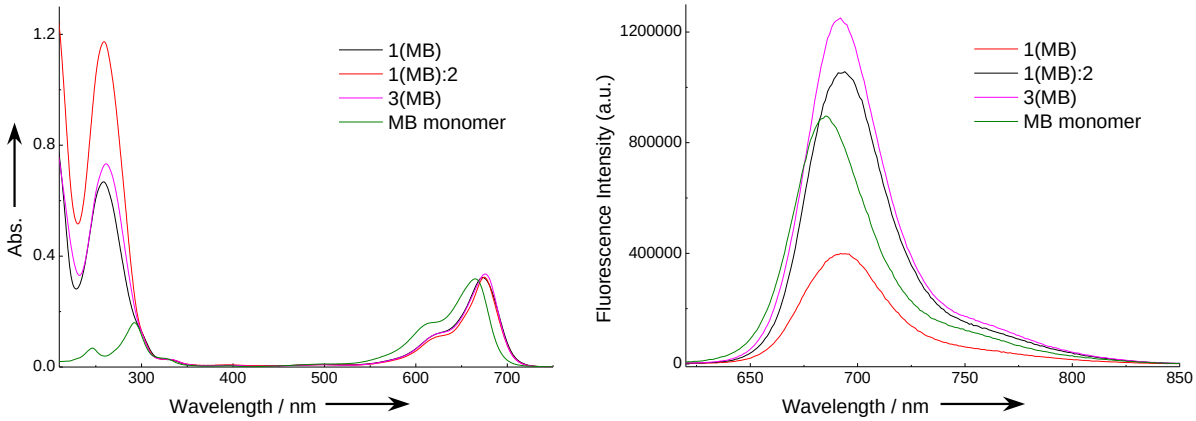

Figure S1. Absorption and fluorescence spectra (λ_{ex} = 600 nm) for MB-monomer and MB-modified DNA. The sample aqueous solution contained 4 μM DNA or MB-monomer, 100 mM NaCl, in 10 mM Na phosphate buffer (pH 8.0).

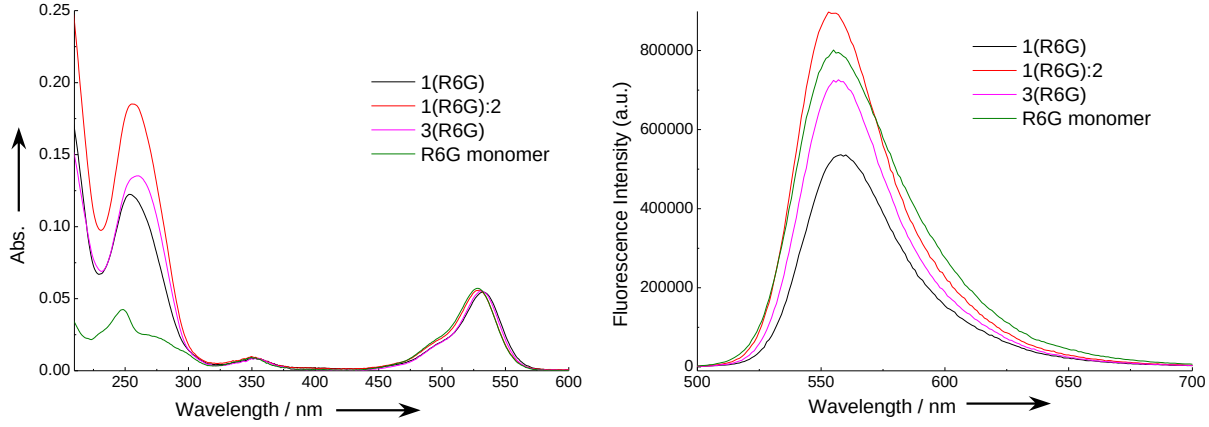

Figure S2. Absorption and fluorescence spectra (λ_{ex} = 480 nm) for R6G-monomer and R6G-modified DNA. The sample aqueous solution contained 0.5 μM DNA or R6G-monomer, 100 mM NaCl, 10 mM $MgCl_2$, 3% PEG-20,000, in 10 mM Na phosphate buffer (pH 7.0).

Melting temperature (T_m) measurement

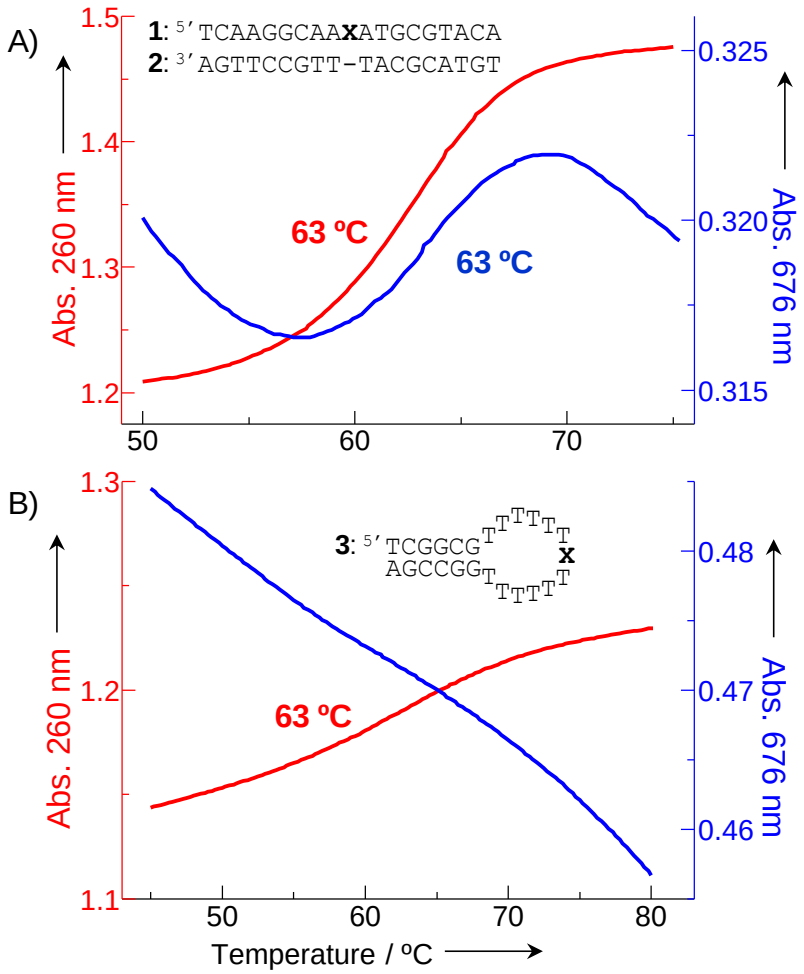

Figure S3. MB π-stacks with neighboring base-pairs in the context of duplex. The UV Melting-temperature (T_m) was measured at 260 nm and 676 nm corresponding to the absorption of nucleobases and MB, respectively. Similar T_m melting values were obtained at two wavelengths in the duplex form (A), while the melting transition at 676 nm was not obvious when MB was placed at the hairpin loop region (B), suggesting that MB is buried in DNA in the duplex form while it is exposed to the solvent in the hairpin form.

References

1. K. Kawai, T. Majima and A. Maruyama, *ChemBioChem*, 2013, **14**, 1430-1433.
2. K. Kawai, E. Matsutani, A. Maruyama and T. Majima, *J. Am. Chem. Soc.*, 2011, **133**, 15568-15577.
3. K. Kawai, M. Hayashi and T. Majima, *J. Am. Chem. Soc.*, 2012, **134**, 4806-4811.
4. K. Kawai, M. Hayashi and T. Majima, *J. Am. Chem. Soc.*, 2012, **134**, 9406-9409.
5. K. Kawai, H. Kodera and T. Majima, *J. Am. Chem. Soc.*, 2010, **132**, 14216-14220.
6. K. Kawai, H. Kodera and T. Majima, *J. Am. Chem. Soc.*, 2010, **132**, 627-630.
7. K. Kawai, H. Kodera, Y. Osakada and T. Majima, *Nat. Chem.*, 2009, **1**, 156-159.
8. S. W. Choi, A. Kano and A. Maruyama, *Nucleic Acids Res.*, 2008, **36**, 342-351.