Development of a fluorescent thymidine analog and construction of orientation-dependent FRET system

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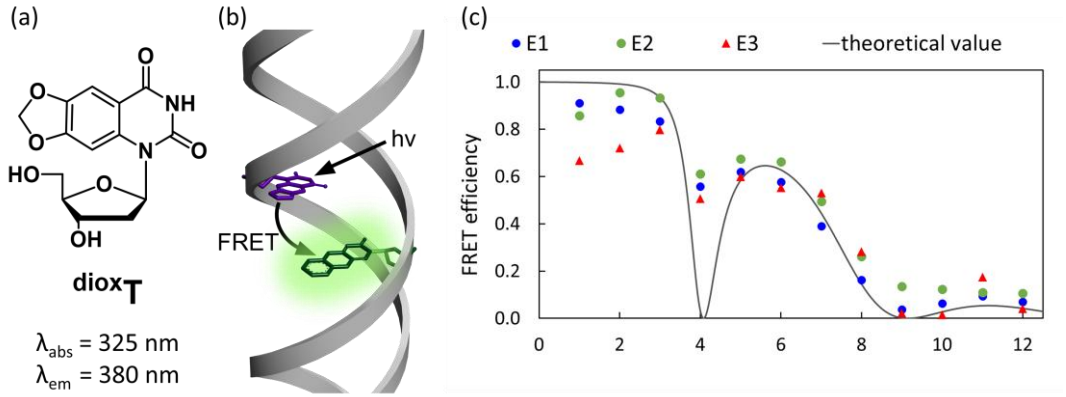
Fluorescent nucleosides are useful tools for investigating the structures and interactions of nucleic acids. As a new bright thymidine analogue and a donor in nucleobase analog FRET pair, we developed ^{diox}T, which consists of a quinazoline derivative attached with 1,3-dioxolane moiety.[1] Thermal denaturation experiment showed that ^{diox}T formed the most stable base pair with adenine. The photophysical property of ^{diox}T was fully investigated considering all combinations of nearest neighboring bases. We found a high quantum yield (0.20) of ^{diox}T when incorporated into double-stranded DNA. This value is relatively high among reported emissive U/T analogues.

Förster resonance energy transfer, FRET has been used as a molecular ruler to monitor the distance between or within biomolecules. A distance- and orientation-dependent FRET system can be a more powerful tool than conventional ones because of higher spatial sensitivity. Such FRET pairs have been developed using DNA scaffolds. We constructed a FRET pair using ^{diox}T,[2] since no FRET pair has used isomorphic U/T analog. We measured experimental FRET efficiencies by steady-state fluorescence and time-resolved fluorescence. The experimental value showed local minimum suggesting the orientation dependency. In comparison of experimental and theoretical FRET efficiency, they showed similar tendency.



(a) Chemical structure of ^{diox}T and its absorption and emission wavelengths. (b) Schematic illustration of our FRET system. (c) Experimental FRET efficiencies based on steady-state fluorescence of donor (E1) and acceptor (E2), and time-resolved fluorescence of donor (E3). Theoretical value (gray).

1) Hirashima, S.; Han, J. H.; Tsuno, H.; Tanigaki, Y.; Park, S.; Sugiyama, H. *Chem. Eur. J.* **2019**, *25*, 9913. 2) Hirashima, S.; Sugiyama, H.; Park, S. *J. Phys. Chem. B* **2020**, *124*, 8794.

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