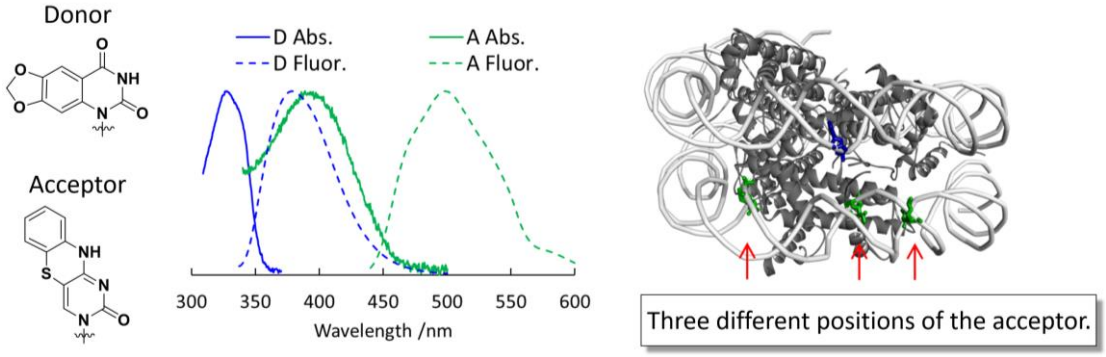
Evaluation of FRET Pair Using Fluorescent Nucleobases in Nucleosome

([1]*Graduate School of Science, Kyoto University*, [2]*Immunology Frontier Research Center (iFReC), Osaka University, Institute for Integrated Cell-Material Science (iCeMS), Kyoto University*) ○Shingo Hirashima,[1] Soyoung Park,[2] Hiroshi Sugiyama,[1,3]

Keywords: Fluorescent nucleic acid, FRET, Nucleosome

FRET is an energy transfer between two molecules and enables real-time observation of biomolecules in solution. In general FRET experiments, bulky fluorophores are attached to target molecules through flexible linkers. While excellent fluorescent properties of such fluorophores, unexpected interactions are concerned by free rotation. Fluorescent isomorphic nucleobases[1] have high structural similarities to native nucleobases and fit within helical structures of nucleic acids. Therefore, fluorescent isomorphic nucleobases can minimize perturbation to target molecules compared with bulky fluorophores. In addition, orientations of fluorescent isomorphic nucleobases are fixed due to hydrogen bonding and stacking interaction.

Recently, our group reported a FRET pair using fluorescent thymidine ($^{\mathrm{diox}}$**T**) and cytidine (**tC**) analogs and their distance- and orientation-dependency.[2] Herein, we demonstrate nucleosomes containing $^{\mathrm{diox}}$**T**–**tC** FRET pair and the evaluation of FRET efficiency based on the positions of the acceptors. Furthermore, we discuss the results combined with theoretical values estimated by molecular dynamics simulations.



Chemical structure and spectra (absorption and fluorescence) of our FRET pair. Schematic illustration of modified nucleosome examined in this study.

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2) Hirashima, S.; Sugiyama, H.; Park, S. *J. Phys. Chem. B* **2020**, *124*, 8794–8800.