
**Supramolecular Chemistry**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

# Time-resolved Laser Spectroscopic Analysis of Multi-step Fluorescence Resonance Energy Transfer on Chromophore Array Constructed by Oligo-DNA Assembly

Yuichi Ohya[a]; Atsushi Nakajima[a]; Tatsuro Ouchi[a]
[a] Department of Applied Chemistry, Faculty of Engineering and High Technology Research Center, Kansai University, Suita, Osaka, Japan

**To cite this Article** Ohya, Yuichi , Nakajima, Atsushi and Ouchi, Tatsuro(2005) 'Time-resolved Laser Spectroscopic Analysis of Multi-step Fluorescence Resonance Energy Transfer on Chromophore Array Constructed by Oligo-DNA Assembly', Supramolecular Chemistry, 17: 4, 283 — 289
**To link to this Article: DOI:** 10.1080/10610270500054101
**URL:** http://dx.doi.org/10.1080/10610270500054101

*Supramolecular Chemistry*, June 2005 Vol. 17 (4), pp. 283–289

Taylor & Francis
Taylor & Francis Group

# Time-resolved Laser Spectroscopic Analysis of Multi-step Fluorescence Resonance Energy Transfer on Chromophore Array Constructed by Oligo-DNA Assembly

YUICHI OHYA*, ATSUSHI NAKAJIMA and TATSURO OUCHI

*Department of Applied Chemistry, Faculty of Engineering and High Technology Research Center, Kansai University, Suita, Osaka 564-8680, Japan*

*Received (in Southampton, UK) 19 January 2005; Accepted 20 January 2005*



**Specific sequential arrangements of three kinds of chromophores separated by regulated distances equaling approximately one pitch of the DNA duplex (34 Å) in non-covalent molecular assembly systems are constructed using chromophore/oligo-DNA conjugates. Vectorial photoenergy transmission along the DNA helix axis by fluorescence resonance energy transfer (FRET) in a sequential chromophore array is observed and analyzed by time-resolved fluorescence spectroscopy and lifetime measurements using a femtosecond pulse laser system. The results suggest a FRET occurs on a picosecond scale between the donor chromophore and the acceptor chromophore through a mediator chromophore via a multi-step FRET over the molecular assemblies (two helical pitches, 68 Å).**

*Keywords*: Chromophore array; Oligo-DNA assembly; FRET; Spectroscopic analysis

## INTRODUCTION

The measurement of fluorescence resonance energy transfer (FRET) reveals a great deal about the structure and dynamics of macromolecules and molecular assemblies. In natural photosynthetic systems, the arrangement of porphyrin derivatives and other chromophores at regulated distances and geometries through non-covalent interactions provides highly efficient photo-induced energy transfers directed at reaction centers [1–6]. Specific chromophores with overlapping excitation and emission spectra that are physically arranged in order of excitation energy level, referred to as a "chromophore array", form a vectorial photoenergy transmission pathway.

The biological tasks of nucleic acids (DNA and RNA) are genetic information storage and propagation. A single chain of poly- or oligo-nucleotide is able to interact specifically with its complementary counter strand through sequence-specific multiple hydrogen bonding. Focusing on this property of nucleic acids, a wide variety of non-covalent binding pairs, binding donors and binding acceptors, with high specificity and stability can easily be provided simply by varying the oligo-DNA sequences. Oligo-DNAs are therefore useful molecular glue for constructing molecular assembly systems and building blocks for constructing nano-sized supramoecular architectures. In fact, high-molecular-weight supramolecular polyassembly systems using complementary oligo-DNAs as binding groups have been reported both by our group [7, 8] and Takenaka *et al.* [9]. Topological DNA assembly systems of various shapes and sizes were reported [10–18]. These characteristics of oligo-DNA facilitate the sequential arrangement of functional molecules [19, 20].

Labeling of oligo- and polynucleotides with fluorescent probes is a very important technique for analysis of DNAs and RNAs. FRET system between probes covalently attached to DNAs and RNAs have been investigated for detection of duplex or triplex DNA formations [21, 22], for structural analyses of DNA and RNA [23–25], and for diagnostic detection of specific genes [26–28]. We previously utilized DNA duplex formation to construct sequential arrangements of the three different chromophores to act as the photo-energy donor, mediator and acceptor [29–31]. Multi-step FRET from donor chromophore to acceptor chromophore

*Corresponding author. Department of Applied Chemistry, Faculty of Engineering, Kansai University, Suita, Osaka 564-8680, Japan. Tel.: +81-6-6368-0818. Fax: +81-6-6339-4026. E-mail: yohya@ipcku.kansai-u.ac.jp

ISSN 1061-0278 print/ISSN 1029-0478 online 
DOI: 10.1080/10610270500054101

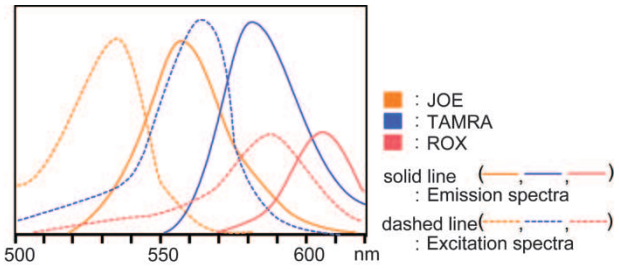


FIGURE 1 Excitation and emission spectra of the chromophores. Excitation spectra are shown by the dotted lines and emission spectra are shown by the solid lines. The excitation wavelengths for emission spectra of JOE, TAMRA and ROX were 532 nm, 561 nm and 588 nm, respectively. Emission wavelengths for excitation spectra of JOE, TAMRA and ROX were 555 nm, 578 nm and 602 nm, respectively.

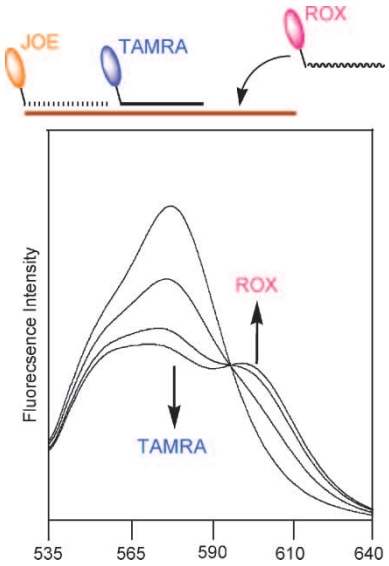


FIGURE 3 Fluorescence spectra for the titration of [mODN + JOE/ODN1 + TAMRA/ODN2] by ROX/ODN3 in 0.05 M Tris–HCl 0.5 M NaCl buffer (pH 7.5), at 5°C. [mODN] = [JOE/ODN1] = [TAMRA/ODN2] = $5.0 \times 10^{-7}$ M, [ROX/ODN3]/[JOE/ODN1] = 0 ~ 1.0. Excitation wavelength: 532 nm.

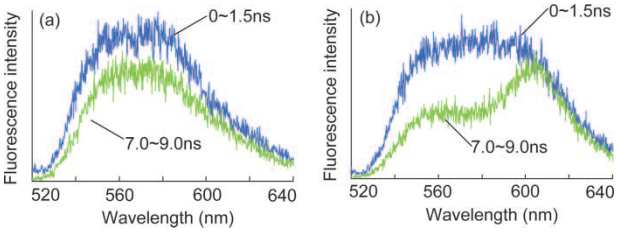


FIGURE 5 Time-resolved fluorescence spectra acquired by integration of the streak images (Figure 4) of the mixture of JOE/ODN1, TAMRA/ODN2, and ROX/ODN3 in the absence (a) and presence (b) of mODN.

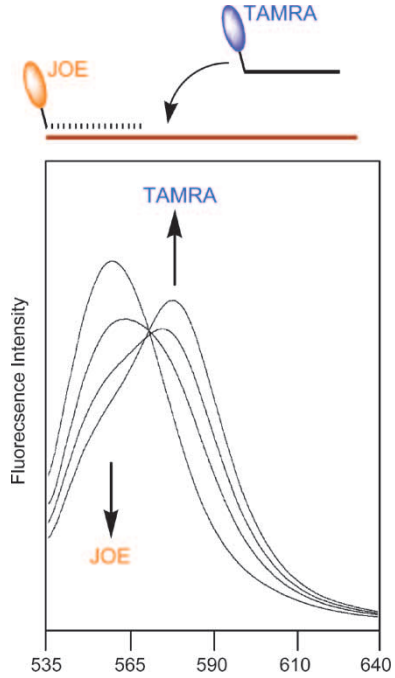


FIGURE 2 Fluorescence spectra for the titration of [mODN + JOE/ODN1] by TAMRA/ODN2 in 0.05 M Tris-HCl 0.5 M NaCl buffer (pH 7.5), at 5°C. [mODN] = [JOE/ODN1] = $5.0 \times 10^{-7}$ M, [TAMRA/ODN2]/[JOE/ODN1] = 0 ~ 1.0. Excitation wavelength: 532 nm.

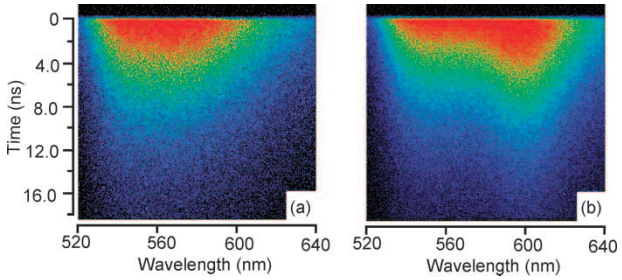


FIGURE 4 Streak images of the fluorescence of the mixture of JOE/ODN1, TAMRA/ODN2, and ROX/ODN3 in the absence (a) and presence (b) of mODN using an excitation wavelength of 513 nm in 0.05 M Tris–HCl 0.5 M NaCl buffer (pH 7.5), at 5°C. [mODN] = [JOE/ODN1] = [TAMRA/ODN2] = [ROX/ODN3] = $5.0 \times 10^{-7}$ M.

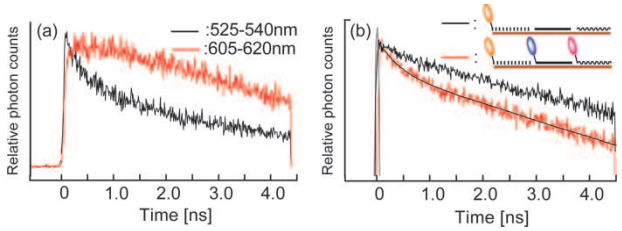


FIGURE 6 (a) Fluorescence decay curves of the mixture of JOE/ODN1, TAMRA/ODN2, and ROX/ODN3 with mODN in the emission wavelength regions of 525–540 nm and 605–620 nm. (b) Fluorescence decay curves of the mixture of JOE/ODN1, TAMRA/ODN2, and ROX/ODN3 with mODN, and the mixture of JOE/ODN1, ODN2, and ODN3 with mODN. The excitation wavelength was 513 nm. The emission wavelength range was 525–540 nm. The concentrations of the each conjugate, ODN and mODN, were $5 \times 10^{-7}$ M.

TABLE I Flourescence decay parameters for chromophore arrays and FRET efficiency.

| | $\tau_D$ [ns] | $a_D$ | $\tau_{ET}$ [ns] | $a_{ET}$ | $\chi^2$ | $\Phi_{ET}$ [%] |
|---|---|---|---|---|---|---|
| | 4.95 | 0.49 | 1.01 | 0.51 | 1.06 | 38 |
| | 4.95 | 0.11 | 1.97 | 0.89 | 1.06 | 6 |
| | 4.95 | 0.31 | 0.88 | 0.69 | 1.03 | 25 |
| | 3.91 | 0.59 | 0.42 | 0.41 | 1.26 | 53 |

$I(t) = a_1\exp(-t/\tau_{ET}) + a_2\exp(-t/\tau_D)$, $a_{ET} = a_1/(a_1 + a_2)$, $a_D = a_2/(a_1 + a_2)$, $\tau_{av} = a_{ET}\tau_{ET} + a_D\tau_D$, $\Phi_{ET} = 1 - \tau_{av}/\tau_D$, I: fluorescence intensity

through one or more mediator chromophore was observed. FRET systems involving three chromophores in a DNA chain have also been reported by other groups [32–36]. In the present study, the vectorial photoenergy transmission behavior, FRET in this case, of a sequential array of three kinds of chromophores as part of an oligo-DNA (ODN) assembly system in aqueous media is observed by time-resolved fluorescence spectroscopy and lifetime measurements using a femtosecond pulse laser system.

The ODN assembly system used in this study consists of a new series of chromophore/ODN conjugates with 10 mer ODNs as complementary-determining regions. Three different chromophores, 6-carboxy-4,5′-dichloro-2,7′-dimethoxyfluoresein (JOE), 6-carbxy-teramethylrhodamine (TAMRA), and 6-carboxy-X-rhodamine (ROX), were employed as the energy donor, mediator, and acceptor, respectively, based on their overlapping excitation and emission spectra (Fig. 1). These chromophores were attached to the 5′-termials of 10 mer ODNs with different sequences. The sequences and structures of the conjugates and matrix ODNs (mODN) consisting of complementary sequences to the conjugates are shown in Chart 1. The sequences of the ODNs were carefully chosen based on several factors. Past research indicated that guanine residue(s) have a fluorescence quenching effect on chromophores attached to ODNs when guanine is located near

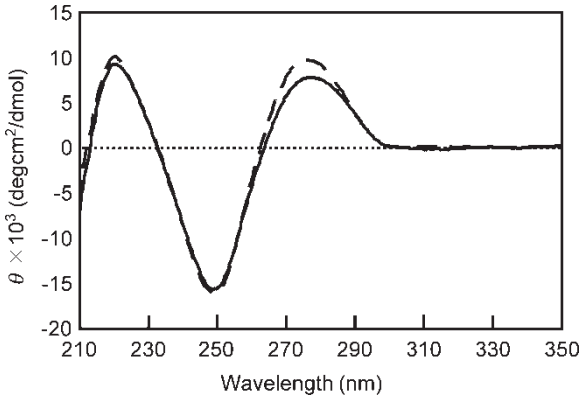


FIGURE 7 CD spectra of equivalent mixture of JOE/ODN1, TAMRA/ODN2. ROX/ODN3 and mODN (solid line), and mixture of ODN1, ODN2, ODN3 and mODN (dashed line) in 0.05 M Tris–HCl 0.5 M NaCl buffer (pH 7.5), at 5°C. Concentration of each conjugate and ODN = $1.5 \times 10^{-6}$ M.


SCHEME 1 Schematic representation for construction of the chromophore array by mixing chromophore/ODN conjugates with complementary mODN.

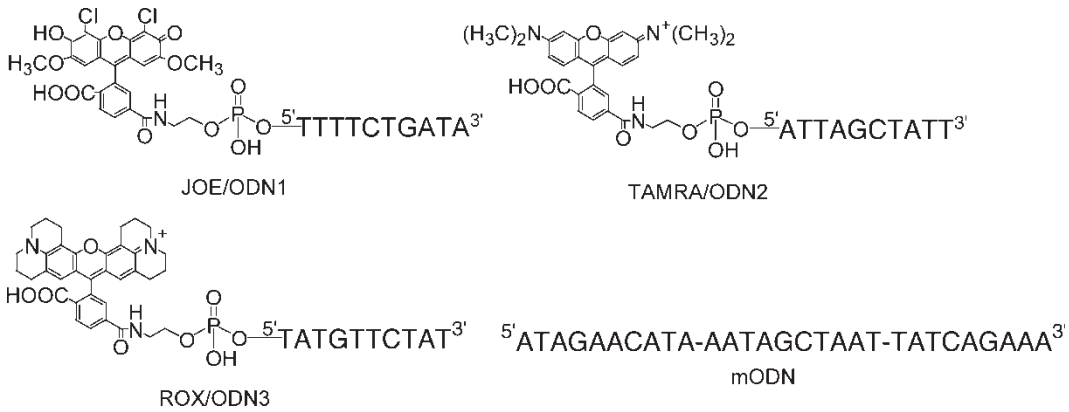


CHART 1 Structures and sequences of the chromophore/ODN conjugates and mODN.

the chromophore [37]. Guanine residues have also been reported to exhibit charge transport abilities via a hole transport mechanism [38, 39]. To avoid such quenching phenomena, only A-T pairs were located near the chromophores (terminal region of the ODNs) in the conjugates. The resulting conjugates were mixed with mODN (30 mer) consisting of complementary sequences for the conjugates in order to construct a sequential JOE-TAMRA-ROX array (chromophore array) in a DNA duplex with 10 residues separating each chromophore component (Scheme 1). The chromophores on the DNA duplex were assumed to be arranged on the same side of the DNA duplex separated by one helical pitch (34 Å).

## RESULTS AND DISCUSSION

### Continuous Light Source Measurements

Before the time-resolved measurements, we studied multi-step FRET behavior of sequential chromophore array by using continuous light source using conventional fluorescence spectrophotometer. First, we investigated single-step FRET behavior between JOE and TAMRA. Figure 2 shows the fluorescence spectra for titration of JOE/ODN1 and mODN by TAMRA/ODN2. Fluorescence spectra were measured at an excitation wavelength of 532 nm ($\lambda_{max}$ of JOE) in 0.05 M Tris·HCl7–0.5 M NaCl buffer (pH 7.5) at 5°C. A schematic illustration of the experimental procedure is also shown in Fig. 2. Quenching of fluorescence from donor chromophores (JOE) and increased fluorescence from acceptor chromophores (TAMRA) was observed on addition of TAMRA/ODN2. These results suggest that single-step FRET from JOE to TAMRA occurred along the DNA duplex. Then, we investigated two-step energy transfer from JOE (donor) through TAMRA (mediator) to ROX (acceptor) on a duplex with a 30 mer mODN. Figure 3 shows the fluorescence spectra of the titration for the complex of JOE/ODN1, TAMRA/ODN2 and mODN by ROX/ODN3. Fluorescence spectra were measured at an excitation wavelength of 532 nm ($\lambda_{max}$ of JOE) in 0.05 M Tris·HCl–0.5 M NaCl buffer (pH 7.5) at 5°C. A schematic illustration of the experimental procedure is also shown in Fig. 3. Quenching of TAMRA and increased fluorescence from ROX were then observed on addition of ROX/ODN3. The fact that obvious fluorescence from TR was observed at an excitation wavelength of 532 nm indicates that the photo-energy transferred from JOE to TAMRA was further transferred to ROX by two-step FRET.

### Time-resolved Measurements

It is important to get the time-scale information about FRET. We can estimate energy transfer efficiency of FRET by lifetime measurement. In order to obtain time-scale information of multi-step FRET of the chromophore array, we measured time-resolved fluorescence spectra by using a femtosecond laser as light source and a streak camera as detector. Figure 4 shows the streak images of the fluorescence of the mixture of JOE/ODN1, TAMRA/ODN2, and ROX/ODN3 in the absence and presence of mODN using an excitation wavelength of 513 nm. Figure 5 shows the time-resolved fluorescence spectra obtained using integrals of 0–1.5 ns and 7.0–9.0 ns from the streak images. In Fig. 5(a), in which mODN was absent, the fluorescence was mainly from JOE (around 560 nm) and partially from TAMRA (around 580 nm). Both the 0–1.5 ns and 7.0–9.0 ns spectra had the same shape. Fluorescence from ROX was not observed. These results indicate that no energy transfer occurred among the chromophores, which was expected, as the conjugates do not interact in this system. On the other hand, in Fig. 5(b), fluorescence from ROX (around 610 nm) was clearly observed at 7.0–9.0 nsec. These results suggest that a two step FRET from JOE to ROX through TAMRA occurred, and that the time-scale of the FRET was faster than the nanosecond scale.

Figure 6(a) shows the fluorescence decay curves of the chromophore array (the mixture of JOE/ODN1, TAMRA/ODN2, and ROX/ODN3 with mODN) in the emission wavelength regions of 525–540 nm and 605–620 nm obtained from the streak image (Fig. 4(b)). The fluorescence from JOE (525–540 nm) rose very quickly and decayed. The fluorescence from ROX (605–620 nm), however, rose gently, reached a maximum after about 500 ps, and then decayed gradually. These results indicate that the FRET from JOE to ROX through TAMRA occurred on the order of 100 ps. Figure 6(b) shows the fluorescence decay curve of the chromophore array (JOE-TAMRA-ROX system) and the fluorescence decay curve of the system without TAMRA and ROX (mixture of JOE/ODN1, ODN2, and ODN3 with mODN). In this chromophore array system, the fluorescence from JOE decayed more rapidly compared with the JOE only system due to the FRET. Decay curves like these were observed in several systems [40, 41], and the lifetimes estimated. The decay curves of the energy transfer systems were fitted by two lifetime components, $\tau_D$, intrinsic to the donor (JOE), and $\tau_{ET}$, attributable to the energy transfer. The results are summarized in Table I. Using these lifetime values, FRET efficiencies, $\Phi_{ET}$, were calculated using the equations shown in Table I [40, 41]. The one step FRET efficiencies from JOE to TAMRA and from TAMRA to ROX were 38% and 53%, respectively. The two-step FRET efficiency from JOE to ROX through TAMRA was estimated to be 25%. This value was in

good agreement with the individual values from each component.

### CD Spectra Measurement

In order to obtain information about the conformation of the assembly, CD spectra were measured for mixtures of chromophore/ODN conjugates and mODN at 5°C. Solid line in Fig. 7 shows a typical example for equivalent mixtures of JOE/ODN1, TAMRA/ODN2, ROX/ODN3 and mODN. Strong negative and positive cotton effects were observed around 250 nm and 280 nm, respectively. This pattern displays the typical characteristics of a B-form DNA duplex and is almost the same as that seen for the equivalent mixture of ODN1, ODN2, ODN3 and mODN (dashed line). The assembly system of the three kinds of chromophore/ODN conjugates with the 30mer mODN therefore displayed a B-form double helical conformation. Moreover, no induced CD was observed in longer wavelength regions, at which the chromophores have absorptions (data not shown). These results mean that the chromophores were not strongly bound by intercalation or groove binding, but exist with freedom of orientation. As the B-form DNA duplex has 10 residues per turn (34 Å), the system was believed to have all chromophores on the same side of the duplex at one-pitch distance (34 Å).

## CONCLUSION

Specific sequential arrangements of chromophores separated by regulated distances of about one pitch of the DNA duplex (34 Å) were constructed by mixing chromophore/ODN conjugates with longer mODN. Time-scale information and the efficiency of the vectorial multi-step FRET from JOE to ROX through TAMRA along the DNA duplex axis were obtained from femtosecond pulse laser spectroscopic analysis. The results suggested that FRET occurred from JOE to ROX over a long distance (two helical pitches, 68 Å) through TAMRA via multiple steps on the order of picoseconds in the molecular assemblies. The information obtained from this system may be useful in constructing a photon-collecting antenna for an artificial photosynthetic system.

## EXPERIMENTAL SECTION

### General Methods

The UV–VIS absorption and fluorescence spectra were recorded on UV-2500PC (Shimadzu, Japan) and F4010 (Hitachi, Japan) spectrophotometers, respectively. CD spectra were measured using a J-600 (JASCO, Japan). MALDI-TOF-MS experiments were performed on an AXIMA CFR (Shimadzu, Japan) [negative mode, matrix: 3-hydroxypicolic acid ($H_2O$/acetonitrile = 7/3)] after samples were treated with 0.1 M diammonium hydrogen citrate solution. Reverse-phase HPLC was carried out using a Toso-8020 system with a TSKgel OligoDNA RP column.

### Materials

Succinimidyl esters of JOE, TAMRA and ROX were purchased from Molecular Probes, Inc. (USA). 5′-End free fully protected oligo-DNAs on solid support (control pore glass, CPG), and non-modified oligo-DNAs were obtained from Hokkaido System Science Co Ltd. (Japan). Aminoethanol, ethyl trifluoroacetate, 1*H*-tetrazole/acetonitrile solution and anhydrous acetonitrile were purchased from Wako Pure Chemical Ind. (Japan). 2-Cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite was purchased from Aldrich Chemical Co. Inc. (USA). Water was purified using a reverse-osmotic membrane. DMF and other organic solvents were purified by usual distillation methods. Other materials were of reagent grade and used without further purification.

### Synthesis of Chromophore/ODN Conjugates

The syntheses of chromophore/oligo-DNA conjugates were performed using 5′-aminoethyl-oligoDNAs and amine-reactive succinimidyl esters of JOE, TAMRA and ROX, according to the methods reported previously with minor midifications [31]. A typical example for the preparation of JOE/ODN1 conjugate is as follows. 5′-Aminoethyl-oligoDNA(ODN1) prepared by the methods reported previously [29] was dissolved in 200 μL of sodium carbonate/bicarbonate buffer (1 M, pH 9) and added to succinimidyl esters of JOE solution in DMF (50 μl, 10 mg·ml$^{-1}$). The reaction mixture was kept in the dark at room temperature for 5 h. The resulting JOE/ODN1 conjugate was purified by gel-filtration chromatography (Sephadex G-25, water) and reverse-phase HPLC (column: TSKgel OligoDNA RP, eluent: 0.1 M-aqueous triethylammonium acetate/acetonitrile, 90/10 to 60/40 gradient) until a single sharp peak was obtained. Identification of conjugates was performed using MALDI-TOF-MS. Attachment of TAMRA or ROX to ODN2 or ODN3 was performed under the same procedures using succinimidyl esters of TAMRA or ROX, respectively, instead of succinimidyl esters of JOE. The identification of the conjugates were carried out by MALDI-TOF-MS spectra [negative mode, matrix: 3-hydroxypicolic acid ($H_2O$/acetonitrile = 7/3)] after samples were treated with 0.1 M diammonium hydrogen citrate solution. Spectral data of the conjugates are available in the supporting information. MALDI-TOF-MS (negative mode, $[M - H]^-$): for JOE/ODN1

calcd 3614.5, found 3615.0; for TAMRA/ODN2 calcd 3549.7, found 3549.1; for ROX/ODN3 calcd 3644.7, found 3644.2.

### Spectroscopic Measurements

Concentration of unmodified ODN was calculated from the UV absorbance at 260 nm (A260), and extinction coefficients were determined using the nearest-neighbor approximation. Concentration of each chromophore/ODN conjugate was determined using UV–VIS absorbance. Conjugate concentration in solution was confirmed by the fact that their hypochromicity with complementary unmodified ODNs was maximal at 1/1 molar ratio. Hypochromicity measurement was performed by monitoring A260 of the solution containing the conjugate and complementary ODN in various ratios. CD spectra were measured on a JASCO J-600 using quartz cells of 0.5-cm path length at 15°C. Concentrations of each conjugate and mODN were $1.5 \times 10^{-6}$ M. Fluorescence measurements were performed on a Hitachi F4010, using a 1 × 1 cm quartz cell. The excitation wavelengths for emission spectra of JOE, TAMRA and ROX were 532 nm, 561 nm and 588 nm, respectively. Emission wavelengths for excitation spectra of JOE, TAMRA and ROX were 555 nm, 578 nm and 602 nm, respectively.

Fluorescence spectra for the mixture of several chromophore/ODN conjugates and complementary mODN were obtained by varying the mixing ratios. The following procedure is an example of the titration of JOE/ODN1 and mODN with TAMRA/ODN2. "Solution A" containing JOE/ODN1 ($5.0 \times 10^{-7}$ M) and mODN ($5.0 \times 10^{-7}$ M), and "Solution B" containing JOE/ODN1 ($5.0 \times 10^{-7}$ M), mODN ($5.0 \times 10^{-7}$ M) and TAMRA/ODN2 ($40.0 \times 10^{-7}$ M) were prepared. The fluorescence spectra of "Solution A" were measured under titration with "Solution B". The final ratio of JOE/ODN1 to TAMRA/ODN2 was 1.0. All fluorescence experiments were performed at 5°C in 0.05 M Tris·HCl buffer (pH 7.5) containing 0.5 M NaCl. The fluorescence intensity of each chromophore in mixed systems containing two or more chromophores was calculated by decomposition of the obtained spectra to the emission spectra of each chromophore.

### Laser Spectroscopic Measurements

In the laser spectroscopic experiments, a pulse at 775 nm was generated by a Ti: Sapphire amplified laser system (CPA-2001, Clark-MXR Inc.). The pulse at 775 nm was changed to a 513 nm pulse by a double-pass single crystal optical parametric amplifier (Vis-OPA, Clark-MXR Inc.). The excitation beam had a repetition rate of 1 MHz, a full width at half-maximum (FWHM) of about 150 fs, and was focused on the front of surface of a 1.0 cm × 0.2 cm quartz sample cell by a lens ($d = 10$ cm). The fluorescence emission was detected by a streak scope (C-4334, Hamamatsu). The instrument response was characterized by a FWHM of about 40 ps. Fluorescence decay profiles and time-resolved fluorescence spectra were acquired using the time-correlated single-photon-counting method (U8167-01, Hamamatsu). For the lifetime measurements, the decay profile was fitted to a single-exponential function (in the case of the chromophore/ODN complex) or a secondary-exponential function (for the FRET systems).

### *Acknowledgements*

A part of this study was financially supported by a Grant-in-Aid for Scientific Research on Priority Areas (#13132207) from The Ministry of Education, Science, Sports and Culture, Japan.

### *References*

[1] McDermott, G.; Prince, S. M.; Freer, A. A.; Hawthornthwaite-Lawless, A. M.; Papiz, M. Z.; Cogdell, R. J.; Isaacs, N. W. *Nature* **1995**, *374*, 517–521.
[2] van Grondelle, R.; Dekker, J. P.; Gillbro, T.; Sundstrom, V. *Biochim. Biophys. Acta* **1994**, *1187*, 1–65.
[3] Moser, C. C.; Dutton, P. L. *Biochim. Biophys. Acta* **1992**, *1101*, 171–176.
[4] Kuhlbrandt, W.; Wnag, D. N. *Nature* **1991**, *350*, 130–134.
[5] Kuhlbrandt, W.; Wnag, D. N.; Fujiyoshi, Y. *Nature* **1994**, *367*, 614–621.
[6] Kuhlbrandt, W. *Nature* **1995**, *374*, 497–498.
[7] Ohya, Y.; Noro, H.; Komatsu, M.; Ouchi, T. *Chem. Lett.* **1996**, 447–448.
[8] Ohya, Y.; Noro, H.; Ouchi, T. *Supramol. Chem.* **2004**, *6*, 157–163.
[9] Takenaka, S.; Funatsu, Y.; Kondo, H. *Chem. Lett.* **1996**, 891–892.
[10] Seeman, N. C. *Nature* **2003**, *421*, 427–431.
[11] Seeman, N. C. *DNA Cell Biol.* **1991**, *10*, 475–486.
[12] Zhang, Y.; Seeman, N. C. *J. Am. Chem. Soc.* **1994**, *116*, 1661–1669.
[13] Chen, J.; Seeman, N. C. *Nature* **1991**, *350*, 631–633.
[14] Winfree, E.; Liu, F.; Wenzler, L. A.; Seeman, N. C. *Nature* **1998**, *394*, 539–544.
[15] Mao, C.; Sun, W.; Seeman, N. C. *J. Am. Chem. Soc.* **1999**, *121*, 5437–5443.
[16] Matsuura, K.; Yamashita, T.; Igami, Y.; Kimizuka, N. *Chem. Commun.* **2003**, 376–377.
[17] Cai, L.; Tabata, H.; Kawai, T. *Appl. Phys. Lett.* **2000**, *77*, 3105–3106.
[18] Maeda, Y.; Tabata, H.; Kawai, T. *Appl. Phys. Lett.* **2001**, *79*, 1181–1183.
[19] Yan, H.; Park, S. H.; Finkelstein, G.; Reif, J. H.; LaBean, T. H. *Science* **2003**, *301*, 1882–1884.
[20] Matuura, K.; Hibino, M.; Yamada, Y.; Kobayashi, K. *J. Am. Chem. Soc.* **2001**, *123*, 357–358.
[21] Cardullo, R. A.; Agrawal, S.; Flores, C.; Zamecnik, P. C.; Wolf, D. E. *Proc. Natl. Acad. Soc. USA* **1988**, *85*, 8790–8794.
[22] Mergny, J. L.; Garestier, T.; Rougee, M.; Lebedev, A. V.; Chassignol, M.; Thung, N. T.; Helene, C. *Biochemistry* **1994**, *33*, 15321–15328.
[23] Murchie, A. I. H.; Clegg, R. M.; von Kitzing, E.; Duckett, D. R.; Diekmann, S.; Lilley, D. M. *J. Nature* **1989**, *341*, 763–766.
[24] Mergny, J. L.; Boutorine, A. S.; Garestier, T.; Belloc, F.; Rougee, M.; Bulychec, N. V.; Koshkin, A. A.; Bourson, J.; Levedev, A. V.; Valeur, B.; Thuong, N. T.; Helene, C. *Nucleic Acids Res.* **1994**, *22*, 920–928.

[25] Morrison, L. E.; Hadler, T. C.; Stols, L. M. *Anal. Biochem.* **1989**, *183*, 231–244.
[26] Tyagi, S.; Kramer, F. R. *Nature Biotech.* **1996**, *14*, 303–308.
[27] Clegg, R. M.; Murchie, A. I. H.; Zechel, A.; Lilley, D. M. J. *Proc. Natl. Acad. Soc. U.S.A.* **1993**, *90*, 2994–2998.
[28] Morrison, L. E.; Stols, L. M. *Biochemistry* **1993**, *32*, 3095–3104.
[29] Ohya, Y.; Yabuki, K.; Komatsu, M.; Ouchi, T. *Polym. Adv. Tecnol.* **2000**, *11*, 845–855.
[30] Ohya, Y.; Yabuki, K.; Tokuyama, M.; Ouchi, T. *Supramol. Chem. 15* **2003**, 45–54.
[31] Ohya, Y.; Yabuki, K.; Hashimoto, M.; Nakajima, A.; Ouchi, T. *Bioconj. Chem.* **2003**, *14*, 1057–1066.
[32] Kawahara, S.; Uchimaru, T.; Murata, S. *Chem. Commun.* **1999**, 563–564.
[33] Horsey, I.; Furey, W. S.; Harrision, J. G.; Osborne, M. A.; Balasubramanian, S. *Chem. Commun.* **2000**, 1043–1044.
[34] Tong, A. K.; Jackusch, S.; Li, Z.; Zhu, H.-R.; Akins, D. L.; Turro, N. J.; Ju, J. *J. Am. Chem. Soc.* **2001**, *123*, 12923–12924.
[35] Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2002**, *124*, 15208–15216.
[36] Heilemann, M.; Tinnefeld, P.; Mosteiro, G. S.; Parajo, M. G.; Van Hulst, N. F.; Sauer, M. *J. Am. Chem. Soc.* **2004**, *126*, 6514–6515.
[37] Ohya, Y.; Yabuki, K.; Ouchi, T. *Supramol. Chem.* **2003**, *15*, 149–154.
[38] Meggers, E.; Micheal-Beyerle, M. E.; Giese, B. *J. Am. Chem. Soc.* **1998**, *120*, 12950–12955.
[39] Giese, B.; Amaudrut, J.; Kohler, A. K.; Spormann, M.; Wessely, S. *Nature* **2001**, *412*, 318–320.
[40] Sessler, J. L.; Wang, B.; Harriman, A. *J. Am. Chem. Soc.* **1995**, *117*, 704–714.
[41] Adronov, A.; Gilat, S. L.; Frechet, M. J.; Ohta, K.; Neuwahl, F. V. R.; Fleming, G. R. *J. Am. Chem. Soc.* **2000**, *122*, 1175–1185.