Change in fluorescence spectra depending on the arrangement of the chromophores in multistep fluorescence resonance energy transfer

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Multistep fluorescence resonance energy transfer in a system containing three chromophores is dependent upon the relative arrangement of the chromophore triad.

Keywords: fluorescence resonance energy transfer, chromophores

Fluorescence resonance energy transfer (FRET)[1,2] is widely used to estimate chromophore separation on a nanometre scale.[3] FRET in a system consisting of a pair of a fluorescent energy donor (D) and acceptor (A) has been extensively studied.

We have already reported sequential-multistep-FRET by three different chromophores, and enhancement of the efficiency of long-range FRET in the systems.[7] It can be expected that the fluorescence spectra of a system possessing three (or more) chromophores will be considerably changed depending on the arrangement of the chromophores. Herein, we report the change in fluorescence spectra in a sequential-multistep-FRET system using a DNA duplex, possessing three different chromophores.

Figure 5 shows the sequences of DNA-oligomers in the present study and the locations of the chromophores. The sequences of oligo-DNA 15- and 10mer are complementary to that of the 25mer. Thus, these oligomers form a typical B-type duplex. We call the 5'-terminals of 25-, 15-, and 10mer position A, B, and C, respectively. Chromophore **1** was introduced at position A, while chromophores **2**, **3** and **4** were introduced at position B or C. For example, **1**–**2**–**3** represents the oligo-DNA duplex possessing chromophores **1**, **2**, and **3** at position A, B, and C, respectively. **X** means that no fluorescent chromophore is introduced at the position(s).

Figure 6A shows the fluorescence spectra of **1**–**2**–**4** and **1**–**4**–**2**, and Fig. 6B shows the fluorescence spectra of **1**–**X**–**X**, **1**–**3**–**4** and **1**–**4**–**3**. In the case of the combination of chromophores **1**, **3** and **4**, change in fluorescence spectra depending on the sequence of the chromophores was not remarkable (Figure 6B), and the fluorescence spectra of **1**–**3**–**4** and **1**–**4**–**3** were very similar to that of **1**–**X**–**X**. So it was impossible to calculate $\Phi_f^{rel}(\mathbf{n})$ in the **1**, **3** and **4** combination. On the other hand, in the case of the combination of chromophores **1**, **2** and **4**, a remarkable change in fluorescence spectra depending on the sequence of the chromophores was observed combination (Figure 6A). It is important to select an appropriate combination of chromophores to observe clearly the difference in fluorescence spectra depending on the arrangement of chromophores, considering the fact that the difference in fluorescence spectra of **1**–**3**–**4** and **1**–**4**–**3** was small.

Table 2 shows $\Phi_f^{rel}(\mathbf{n})$ of each chromophore. It is expected that smooth energy transfer occurs in **1**–**2**–**4** compared to **1**–**4**–**2**, because overlap integration between **1** and **2** is larger than between **1** and **4**. However, the emission intensity of chromophore **4** of **1**–**4**–**2** was higher than that of **1**–**2**–**4**, because chromophore **4** should be excited *via* direct energy transfer from chromophore **1** in the former.

Techniques used: Fluorescence, UV-Vis

References: 9

Figure 1 Expected energy transfer manner in conventional FRET system (A and B) and in sequential-multistep-FRET (C and D).

Figure 2 Structures of the chromophores.

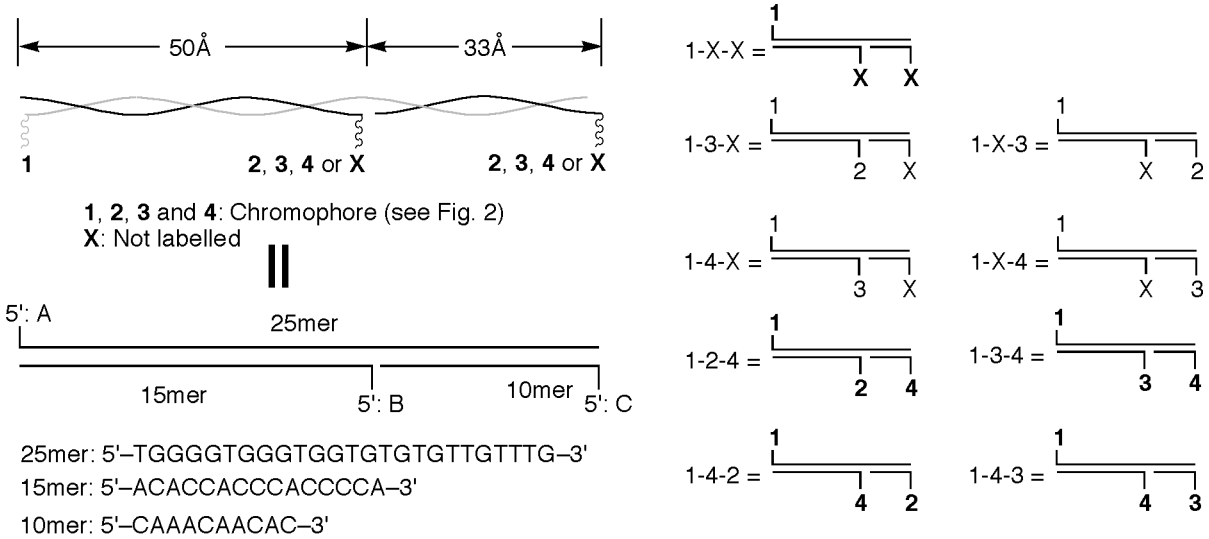

Fig. 5 DNA Sequences and chromophore introduced positions

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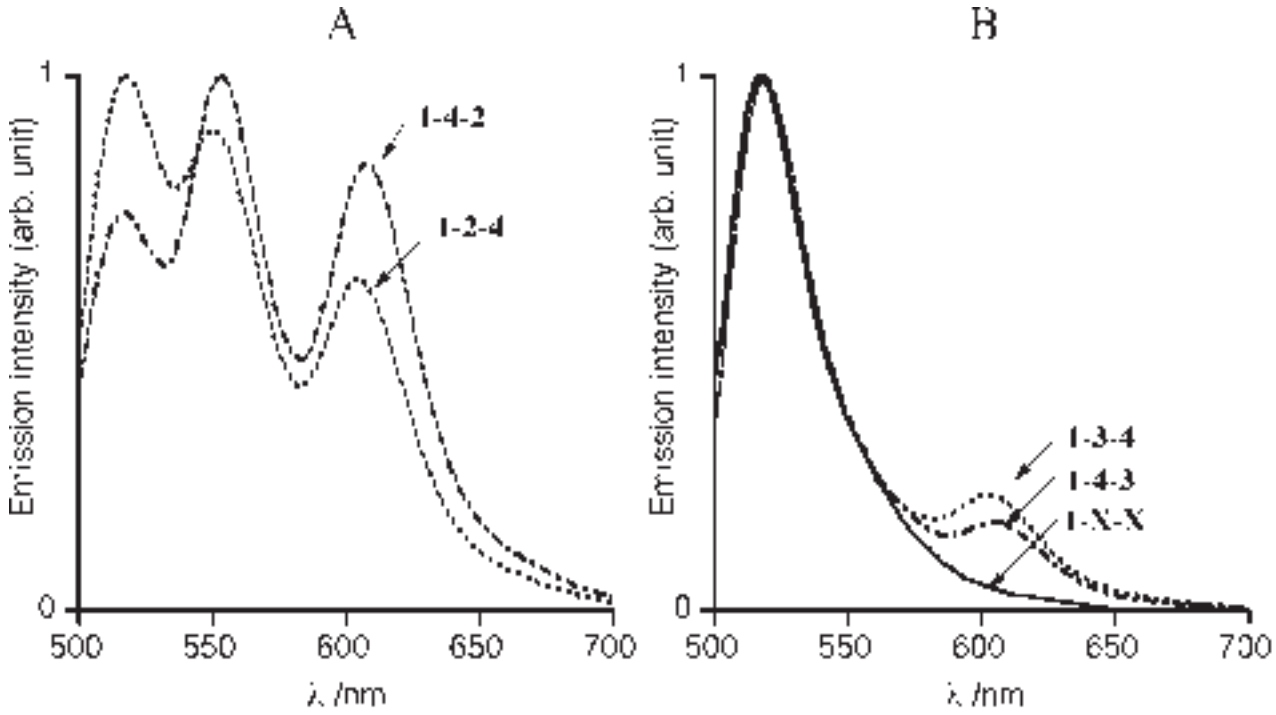

Fig. 6 Fluorescence spectrum of each sequence

Table 2 $\Phi_f^{rel}(\boldsymbol{n})$ of each sequence of chromophore.

	Observed			Normalized[a]		
Sequence	**1**	**2**	**4**	**1**	**2**	**4**
1–2–X	0.57	0.54	–	0.51	0.49	–
1–X–2	0.85	0.36	–	0.73	0.27	–
1–4–X	0.58	–	0.36	0.62	–	0.38
1–X–4	0.90	–	0.10	0.90	–	0.10
1–2–4	0.51	0.29	0.16	0.53	0.30	0.17
1–4–2	0.37	0.42	0.22	0.37	0.41	0.22

[a]Sum of each $\Phi_f^{rel}(\boldsymbol{n})$ in the sequence was adjusted to 1.

Figure 3 Excitation (normal line) and emission (dashed line) spectra of each chromophore normalized at maximum.

Figure 4 Structure of chromophore introduced 5′-terminal of oligo-DNA.

Figure 5 DNA sequences and chromophore introduced positions.

Figure 6 Fluorescence spectrum of each arrangement.

Figure 7 Expected energy transfer manner in branched-FRET system in Ref. 9.

Table 1 Wavelength of fluorescence (Em_{max}, nm) and excitation maximum (Ex_{max}, nm) and extinction coefficient at wavelength of absorption maximum (Ec, l/mol•cm) of the chromophores in the DNA duplexes.

Table 2 $\Phi_f^{rel}(\mathbf{n})$ of each sequence of chromophore.

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