Application Note

Observation of Förster Resonance Energy Transfer (FRET) phenomenon using complementary Oligonucleotide binding

KEYWORDS

Fluorescence Spectrometer, Förster Resonance Energy Transfer (FRET), Oligonucleotide

INTRODUCTION

FRET(Förster resonance energy transfer) is an energy transferring phenomenon that occurs by resonance when two fluorescent substances are close enough. It is widely used in the field of physics, chemistry [1] and especially more in the biology to search for the *in vivo* interaction between the proteins or the genes such as DNA and RNA.

Among these two fluorescent substances, the one that transfers an energy is called 'donor' and the other one that receives an energy is called 'acceptor.' Excitation/emission wavelength of donor is shorter than the acceptor's. There are two major preconditions of FRET occurrence. First, the emission spectrum of the donor must overlap the excitation spectrum of the acceptor. Second, the distance between the donor and the acceptor should be close (typically 10Å~100Å[2]).

When two fluorescent substances are far from each other and with the light at the excitation wavelength of the donor, the donor generates a strong fluorescence, while the acceptor generates a weak fluorescence (Fig. 1 (a)). On the other hand, when they are close, the donor in the excited state transfer its emission energy to the acceptor, causing the donor generates a weak fluorescence, while the acceptor generates strongly[3] (Fig. 1 (b)).

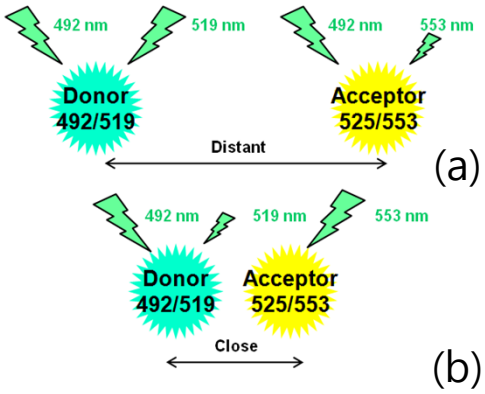

Fig. 1. Diagram of the FRET

Oligonucleotide, a genetic material that has been used in this experiment, is a short nucleic acid polymer, typically with fifty or fewer bases. It can be synthesized artificial sequence and be used in various fields such as Polymerase Chain Reaction (PCR), DNA & RNA sequence and structure analysis, gene expression profiling.

In this experiments, a pair of oligonucleotide with 10 complementary sequences has been used. At their 5' ends, Alexa Fluor® 488 were labeled as a donor and Alexa Fluor® 532 as an acceptor. (Fig. 2)

Before the donor and the acceptor are mixed, both the donor and the acceptor show their own fluorescent characteristics. After the mixing, two oligonucleotides hybridize and become the distance of approx 35Å, which means that the second precondition of the FRET is formed.

The present study aimed to verify the first preconditions of FRET occurrence using Wave Scan mode in FluoroMaster Plus Software and observe the change in fluorescence intensity of donor & acceptor.

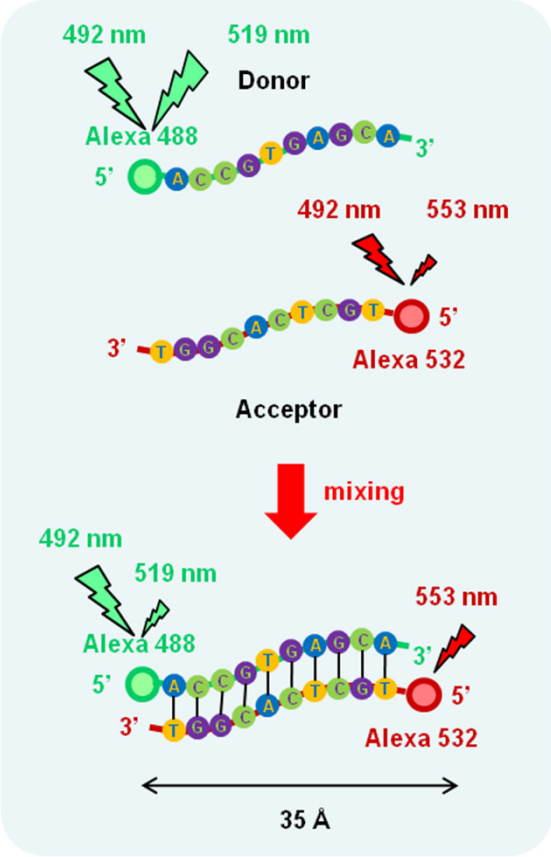

Fig. 2. FRET Phenomenon using the oligonucleotides

REAGENT & APPARATUS

1. Fluorescence Spectrometer (FS-2)
2. Donor Oligonucleodtide
   5′ – (Alexa 488) – ACCGTGAGCA – 3′
3. Acceptor Oligonucleodtide
   5′ – (Alexa 532) – TGCTCACGGT – 3′
4. Tris-EDTA Buffer Solution (pH 8.0)
5. NaCl (ACS reagent, ≥99.0%)
6. 10ml Plastic Tubes
7. Fluorescence cell

PROCEDURE

1. Two plastic tubes are filled with 10 ml Tris-EDTA buffer (pH 8.0). Dissolve NaCl in buffer to make 172mM (donor) and 190mM (acceptor) solution.
2. Dissolve the donor and the acceptor oligonucleotide in each buffer to make 900nM.
3. Measure the excitation/emission spectrum of the donor and acceptor reagent with the Wave Scan mode.
4. Scan emission spectrum of the donor and acceptor at 492 nm excitation wavelength for comparing between before and after FRET. (Fig. 3)
5. Mix the donor & acceptor reagent and incubate for 5 min at the room temperature. Then scan emission spectrum under the same condition.

INSTRUMENT PARAMETER

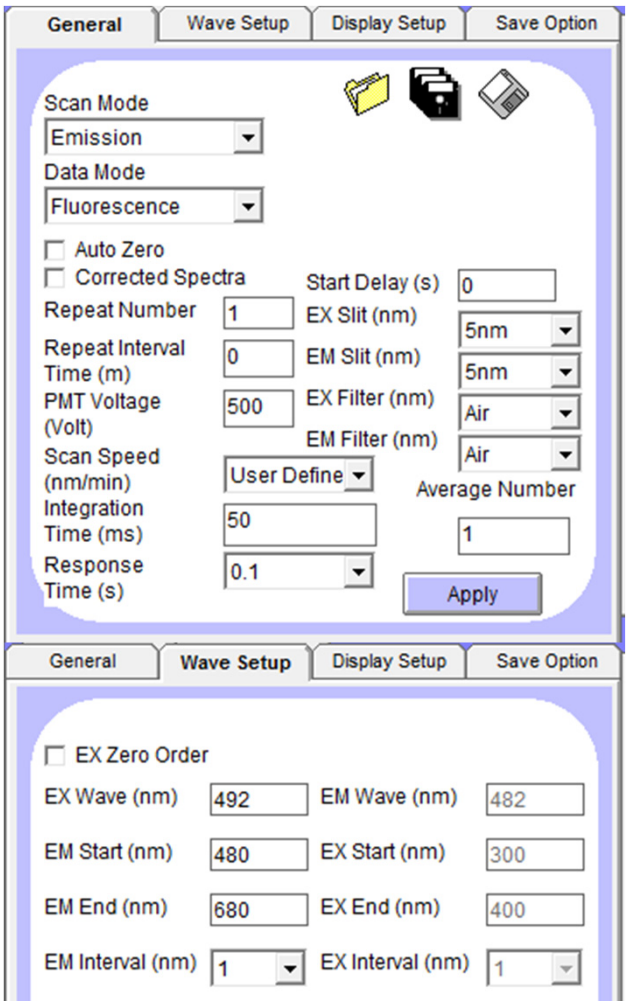

Fig. 3. Measurement condition of the Wave Scan mode

RESULT

Fig. 4 (a), (b) is excitation/emission spectrum of the donor and acceptor sample, measured by the Wave Scan mode. As shown in Fig. 4 (c), two graphs were combined into a single graph to examine the similarity of the emission spectrum of the donor and the excitation spectrum of the acceptor. The combined spectrum verifies that the it is widely overlapped, which is a sufficient condition of FRET occurrence.

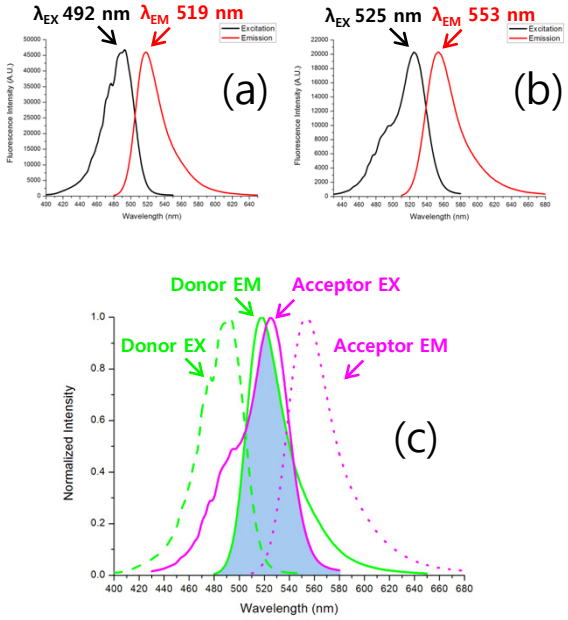

Fig. 4. Spectrum of the Donor sample (a) & acceptor sample (b), and combined spectrum of the two sample (c)

Lastly, FRET phenomenon was verified through the spectrum. As shown in Fig. 5, the fluorescence of the donor is much stronger than the acceptor's before mixing them. But after mixing, the intensity of the acceptor increase, whereas the donor's decrease. As a result, the ratio of intensities reserved by the energy resonance transfer,

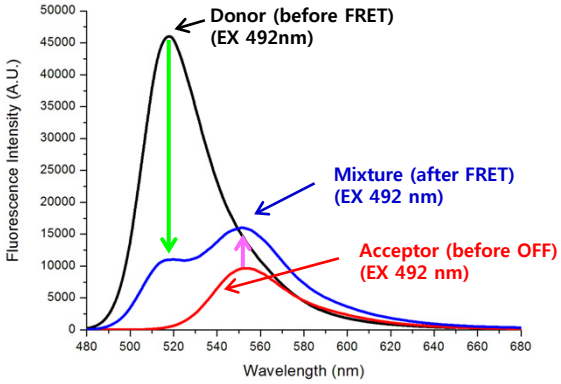

Fig. 5. The change in fluorescence intensity between before/after FRET

CONCLUSION

The SCINCO's FS-2 and the Wave Scan mode in FluoreMaster Plus Software give excitation/emission spectrum of the labeled oligonucleotide with fluorescent probe, and verify the preconditions of FRET occurrence. Furthermore, they assist you to observe Förster resonance energy transfer by tracking the change in fluorescence intensity of the donor & acceptor.

REFERENCE

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