공초점 현미경을 이용한 단일 분자 형광 공명 에너지 전이현상 연구

Single-molecule Detection of Fluoresence Resonance Energy Transfer using Confocal Microscopy

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Fluorescence Resonance Energy Transfer (FRET) is an energy transfer process between two fluorescent dye molecules. It has received great interest for biological applications due to its high sensitivity to the change of the distance between the dyes[(1)]. FRET technique has a spatial resolution of sub-nanomerter, and can be used as a prove of inter- and intra-molecular dynamics of biological macromolecules such as protein conformational change, enzyme-substrate reaction, and RNA folding[(2)]. Determination of FRET efficiency in ensemble measurement is, however, not straightforward because of the difficulty in distinguishing FRET pair from unpaired or unlabeled dyes that gives huge background. By contrast, single-molecule detection provides a method that are free from this obstacle since it can only collect the signal from one single FRET pair.

Here we demonstrated single-molecule detection of FRET from single dye pairs attached to a DNA (IDTDNA) using confocal microscopy. To give a nanometer scale distance between the dye pair, we used a partial duplex DNA consisting of a poly(deoxythymidylic acid) [poly(dT)] single strand DNA attached to a double strand DNA of 18 base pairs (Figure 1-a). As a donor, Cy3 was attached at the 3'-tail of the partial duplex DNA and an acceptor Cy5 was located at the junction of the dsDNA and 3' single strand tail, so that the distance between the donor and acceptor could be manipulated by the number of the deoxythymidylic acids (dT)n. A confocal microscope built around a commercial inverted microscope (Olympus IX51) was used to achieve high S/B ratio. A 532 nm laser (CrystaLaser) beam was sent through an objective lens and focused on the surface of a coverslip in which the partial duplex DNA was attached via BSA-netravidin-biotin linker (Figure 1-b). Fluorescence signals from the donor and acceptor were collected by the same objective lens and separated spectrally by a dichroic mirror. Before the fluorescence were focused at one end of the optical fiber to be guided to avalanche photodiodes (APD), emission filters for Cy3 and Cy5 were inserted to block the scattered laser light. Each single photons measured by the APDs were converted to electrical pulses and counted by commercial counting board (National Instruments). To find DNA molecules attached on the surface of the coverslip, we scanned the sample with piezo-electric controlled translation stage. All measurements are performed at room temperature and 10 mM of Tris buffer with pH 8.0 was used in the presence of 30 mM of Na^+ ion. To avoid photobleaching of the dyes, we added oxygen scavenging system consisting of 1 mg/ml glucose

oxidase (Sigma), 0.8 % (w/v) dextrose (Sigma), and 0.04 mg/ml catalase (Roche). Introducing 1% 2-mercaptoethanol (Sigma) in buffer solution helped quenching of triplet states of dyes.

Shown in Figure 1-c is a scanned DNA image. A representative time trajectory of fluorescence intensities and FRET efficiency obtained from one DNA molecule (n=12) is in Figure 1-d. Initially, the fluorescence of the acceptor was higher than that of the donor to yield a FRET value of ~0.65. Sudden drop of fluorescence intensity in the acceptor channel to its background level at 16 seconds is associated with an anti-correlated increase of the donor fluorescence indicating photobleaching of the acceptor dye. Similarly, photobleaching of the donor molecule was observed at 26 seconds. From these single photobleaching events, we could distinguish a single FRET pair from multi-labelled molecules that showed multiple photobleaching events. Histograms of FRET efficiency from measured single FRET pairs pulled out a peak at around 0.7 for $(dT)_{12}$ and 0.22 for $(dT)_{30}$, respectively.

In summary, we have successfully measured FRET from single donor-acceptor pairs using confocal microscopy. Single photobleaching events were clearly shown in FRET time trajectory, which can be used as a beacon of a single molecule. As the single-molecule FRET technique provides real time observation of inter- and intra molecular dynamics of macromolecules in nanometer scale, we expect that the technique can be used for biological applications such as identifying short-lived state during enzyme activity.

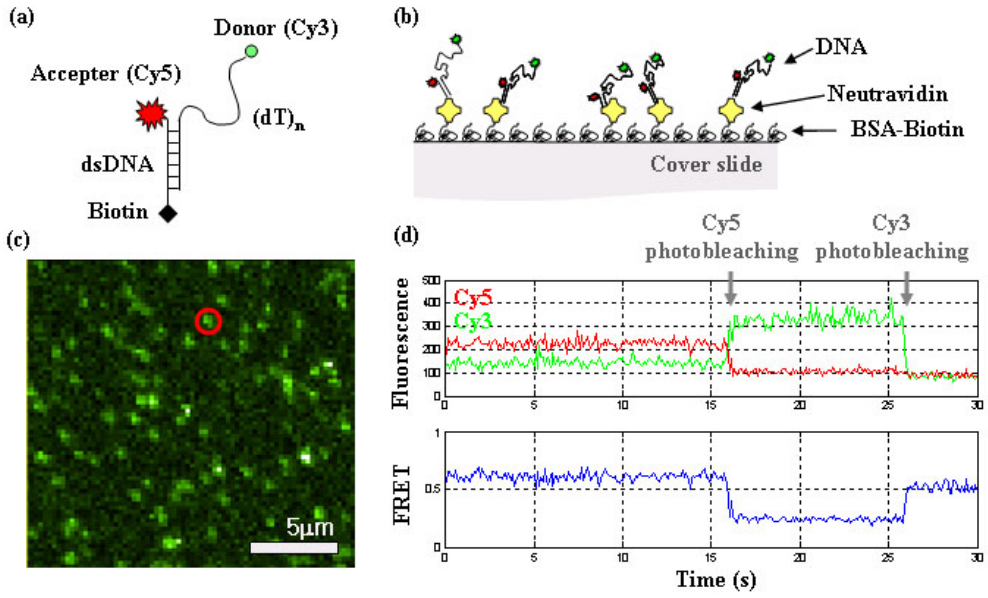

Figure 1. (a) The structure of the partial duplex DNA labeled with Cy3 and Cy5. (b) The partial duplex DNA was attached to cover slide via biotin-Nuetravidin-BSA linker. (c) Scanned single molecule image. (d) A representative single molecule time trajectory of fluorescence intensity and FRET efficiency.

1. T. Forster, "Intermolecular energy migration and fluorescence", Ann. Phys. 2, 55-75 (1948).
2. P. V. Cornish, and T. Ha, "A survey of single-molecule techniques in chemical biology", ACS Chem. Biol. 2, 53-61 (2007).