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Development of a Fluorescent Probe Providing Nonlinear Response through Intramolecular Electron Transfer

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In recent high-resolution fluorescence microscopies, the key to breaking the diffraction limit relies on the reaction of the fluorescent probe. STED and SAX microscopy utilize the nonlinear fluorescence emission of the probe against excitation rate caused by saturation effect [1, 2]. Localization microscopy uses a photo-switchable fluorescent dye [3]. Here, we propose a fluorescent probe providing nonlinear fluorescence response through intramolecular electron transfer, which can be used to achieve high spatial resolution using typical confocal microscopy. The nonlinear fluorescence response by the probe gives fluorescence emission from a region smaller than the diffraction-limited light focus, thereby improving the spatial resolution.

The design of the fluorescent probe is based on two electron donors (D) and one electron acceptor (A), resulting to the structure of "D-A-D". The nonlinear response of D-A-D can be understood from its hypothetical energy diagram (Fig. 1a). The nonlinear reaction is realized by energy transition following paths (i)~(iv). When one of the donors is excited by an incident photon (i), a charge-separated state is formed through intramolecular electron transfer without fluorescence emission (ii). If the other donor is then excited by another incident photon under this charge-separated state (iii), the probe finally emits a fluorescence photon (iv). The energy transition through the electron transfer after simultaneous excitation of both donors shown as (ii')~(iii') is another candidate path for the nonlinear reaction. Hence, this probe requires two incident photons to emit one fluorescence photon. This 2nd order nonlinear reaction process can be induced with a continuous wave excitation.

A probe was developed, which consists of BODIPY as donor and a nitro benzene as acceptor, and aptly named "Nitro-bis BODIPY" to show the proof of the concept of the nonlinear fluorescence probe (Fig. 1b). The 2nd order nonlinear fluorescence response of Nitro-bis BODIPY was measured using a confocal microscope with 488 nm continuous wave excitation (Fig. 1c). While normal BODIPY showed a linear reaction (slope 1) with increasing excitation intensity, Nitro-bis BODIPY showed 2nd order nonlinear emission (slope 2). The fluorescence response of Nitro-bis BODIPY is consistent with simulation results based on the energy diagram shown in Fig. 1a. Finally, we performed fluorescence imaging of fixed HeLa cells using the Nitro-bis BODIPY and compared it with normal BODIPY. The spatial resolution of each stained part of the cells was clearly higher in the case of Nitro-bis BODIPY than in BODIPY, confirming the improvement in spatial resolution of our designed probe.

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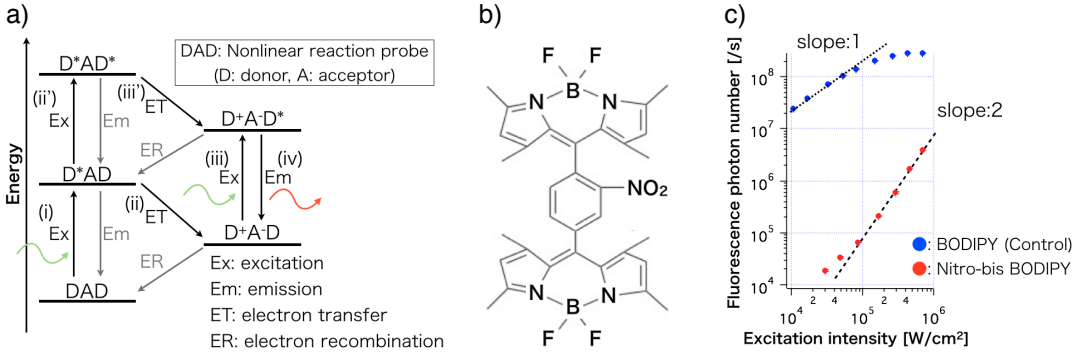

Figure1 a) The energy diagram of the proposed fluorescent probe. b) The molecular structure of Nitro-bis BODIPY. c) A fluorescence response of Nitro-bis BODIPY.