# Development of highly emissive NPs for tracking intracellular dissolution of organic NPs

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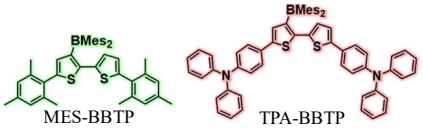
**[Introduction]** Thanks to their high drug loading capacity, carrier-free nanodrugs composed of only water-insoluble compounds exhibit an outstanding potential as a next-generation drug delivery strategy. However, their intracellular behavior and dynamics are still unknown, hindering their progress towards clinical application. One of the critical questions is how water-insoluble nanodrugs degrade in the cells, where water is the main component. Through fluorescence-based strategy, it has been difficult to follow their degradation process from nanoparticles (NPs) to molecules, because common fluorescent probes are quenched in the solid state. In contrast, aggregation-induced emission probes which emit fluorescence in its aggregate/NPs form are quenched in the solution state. In this study, we utilized 3-boryl-2,2'-bithiophene-based compounds[1)] (BBTP) (Fig. 1) which exhibits strong fluorescence in both solid- and solution states as the model probes. Highly emissive NPs was developed for the intracellular investigation of NPs degradation process.

**[Fabrication of FRET NPs]** FRET (Förster Resonance Energy Transfer) is a useful tool for monitoring the integrity/dissociation of NPs as the energy transfer efficiency strongly depends on the distance between donor and acceptor compounds (1-10 nm). FRET NPs of MES-BBTP (doner) and TPA-BBTP (accepter) were fabricated by co-precipitation (Fig. 2a). The emission spectra of FRET NPs and the two compounds in the THF solution are shown in Fig. 2b. The peak of 590 nm derived from TPA-BBTP was only confirmed in FRET NPs dispersion (intact NPs: FRET-ON), while the peak of 500 nm derived from MES-BBTP was confirmed in THF solution (dissolved molecules: FRET-OFF).




MES-BBTP ($\lambda_{EM}$ = 500 nm)　　TPA-BBTP ($\lambda_{EM}$ = 590 nm)

Fig. 1 Chemical structure of 3-boryl-2,2'-bithiophene-based compounds (BBTP).

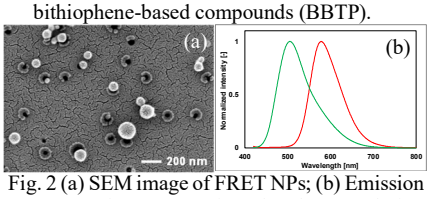



Fig. 2 (a) SEM image of FRET NPs; (b) Emission spectrum of FRET NPs in red and THF solution of MES-BBTP and TPA-BBTP in green.

**[Cell imaging]** To investigate intracellular behavior of FRET NPs, HeLa cells were incubated for 2 h with FRET NPs. At 0 h, the TPA-BBTP emission in red (FRET-ON) was observed (Fig. 3a), while a dominance of MES-BBTP emission in green (FRET-OFF) was observed at 48 h (Fig.3b). These results imply that FRET-NPs entered cells as intact particles (FRET-ON), then followed by particles dissolution (FRET-OFF) in the intracellular environment.

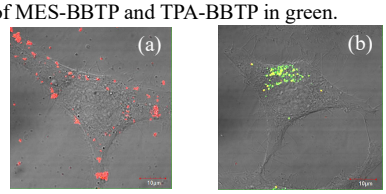



Fig. 3 CLSM images of HeLa cells incubated with FRET-NPs at 0 h (a), 48 h (b), Green (420 nm-500 nm). Red (600 nm-700 nm)

**[Conclusion]** The newly fabricated FRET NPs showed high fluorescence intensity in both solid- and solution states, enabling the monitorization of particles state inside cells. We are currently investigating the dissolution mechanism of NPs and this topic will be discussed.

1) A. Wakamiya et al., Angew. Chem., Int. Ed., 46 (2007), 4273-4276.