Single molecule analysis and diagnosis based on triplet-triplet energy transfer rate measurements

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Abstract

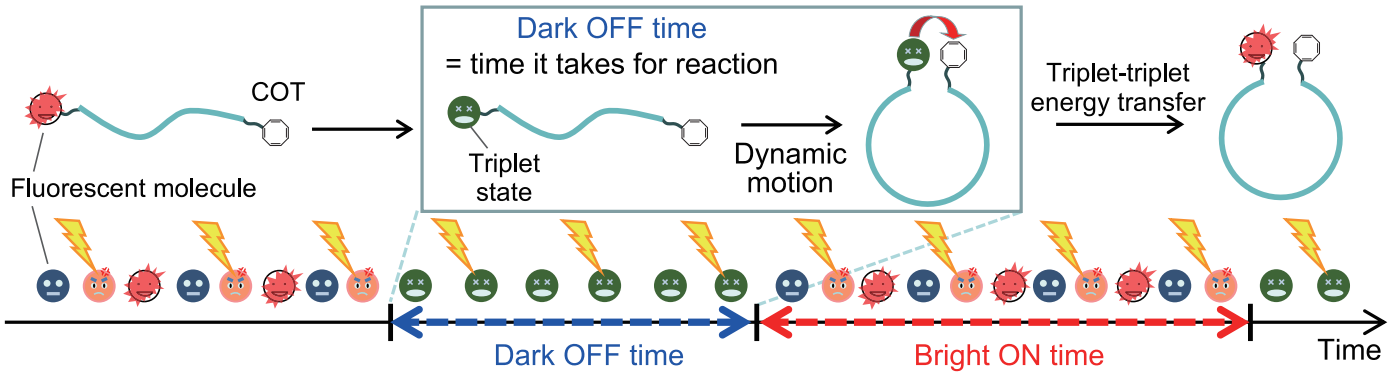
Tracking triplet-triplet energy transfer (TTET) kinetics between fluorescent molecule and cyclooctatetraene (COT) by monitoring fluorescence blinking provides a powerful method to access biomolecular dynamics in the time range of μs to ms and detect miRNAs at the single molecule.

Background & Results

The kinetics of photo-induced chemical reaction through its singlet-excited state S_1 can be easily determined by fluorescence lifetime measurements. However, due to the short lifetime of S_1 (10^{-8} seconds), it can only access reactions and dynamic movements that occur in a very short time. On the other hand, the triplet excited state T_1, which is formed from S_1 through intersystem crossing, has a relatively long lifetime (~10^{-3} s), and thus T_1-based kinetics can be used to investigate reactions and dynamic motions that occur on time scales about 10^5 times wider than those using S_1. TTET is one of the most useful T_1-mediated reactions and has been used to probe the dynamics of biological macromolecules in the time range of ~10^{-3} s. However, T_1-based reactions have traditionally been measured by transient absorption measurements using laser flash photolysis, which requires a considerable amount of sample (more than 1 nmole), making it difficult to analyze valuable samples that are difficult to prepare in large quantities. In this study, we focused on the fact that in single-molecule fluorescence measurements, the lifetime of T_1 can be measured as the duration of the dark OFF state (τ_{OFF}) while the fluorescence is blinking. We have used COT as both a triplet acceptor and a photo-stabilizer to control and observe blinking at the single molecule level. Using DNA as a platform, we have shown that triplet blinking of the fluorescent molecule ATTO 647N can be controlled by the collision reaction between COT. By attaching COT and ATTO 647N to a molecular beacon type probe, we were able to detect miR-155, a model biomarker, at the single molecule.

Significance of the research and Future perspective

Förster resonance energy transfer (FRET), a singlet-singlet energy transfer process, occurs inversely proportional to the sixth power of the distance between the two fluorescent molecules. Therefore, FRET occurs over long distances (1-10 nm) and can be used to study dynamics that induce large distance changes between two fluorescent molecules, while it is not sensitive to local motion over small distances. On the other hand, TTET measurements requires direct Van der Waals contact between ^{3}ATTO 647N* and COT. Consequently, it can be applied to the dynamics of biomolecules with a small distance change and offers more direct information, which is distinctive from that obtained by FRET. Serving as a complementary method to FRET, the present method is a versatile and powerful tool for accessing biomolecular dynamics and detecting biomarkers at the single molecule level.



Schematic representation of fluorescence blinking controlled by triplet formation and triplet-triplet energy transfer.

Patent

Treatise Xu, Jie; Fujitsuka, Mamoru; Kawai, Kiyohiko et al. Control of Triplet Blinking Using Cyclooctatetraene to Access the Dynamics of Biomolecules at the Single-Molecule Level. Angewandte Chemie, International Edition. 2021; 60(23): 12941-12948. doi: 10.1002/anie.202101606

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