

Single molecule analysis and diagnosis by monitoring fluorescence blinking

Kiyohiko KAWAI

Department of Life Science and Technology, Tokyo Institute of Technology
Email: kawai.k@bio.titech.ac.jp

In order to achieve an ultra-sensitive analysis, one strategy would be to focus on an analytic method that relies on the properties of molecules that become highlighted when we look at molecules at the single-molecule level. In our recent studies, we have focused on the phenomenon so called fluorescence blinking. Fluorescent signals from a single fluorophore often blink, reflecting time-dependent fluctuations between bright "ON" and dark "OFF" states.

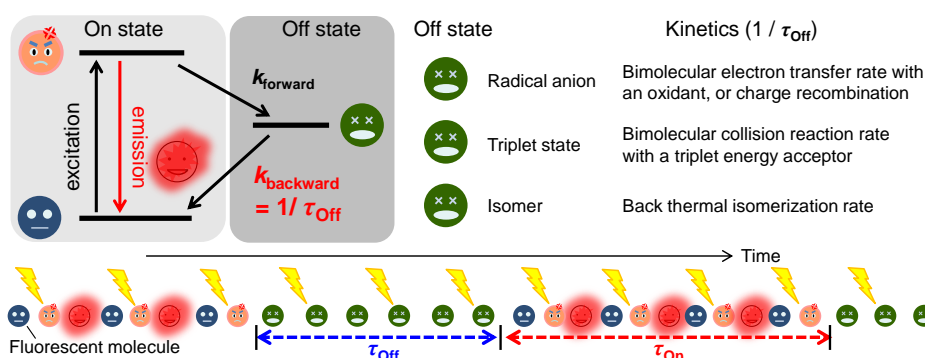


Figure 1: Schematic representation of KACB method.

During the repetitive cycles of excitation and emission, fluorescent molecules may occasionally enter non-fluorescent off states, such as: (a) the reduced or oxidized state triggered by photo-induced electron transfer, (b) the triplet state resulting from intersystem crossing, (c) the isomerized state formed by photo-triggered *trans-cis* isomerization as typically seen in cyanine dyes. Reversible formation of such off states causes a fluorescence blinking. The kinetics of a chemical reaction concomitant with blinking can be followed by the duration of the ON state (τ_{ON}) and that of the OFF state (τ_{OFF}). Based on the understanding of factors that affect the blinking, single fluorescent molecules can serve as reporters of their local microenvironment. We developed a method, termed **K**inetic **A**nalysis based on the **C**ontrol of fluorescence **B**linking (KACB). The blinking kinetics or patterns were controlled to reflect the microenvironment changes around the fluorophore. In this study, KACB method was adapted for detection of target DNA and RNA, and for investigation of antigen-antibody interactions at the single molecule level.

References:

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Research URL: <https://sites.google.com/view/fluorescence-blinking/research?authuser=0>