TWO-PHOTON FLUORESCENCE CORRELATION SPECTROSCOPY AND ITS APPLICATION IN THE INTERACTIONS OF BIOMOLECULES

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Two-photon fluorescence correlation spectroscopy (TPFCS) is a sensitive method that measures the fluctuations of fluorescence signals with two-photon excitation to obtain information of chemical kinetics, diffusion properties and concentrations, as well as molecular dynamics, mobility, and interactions at the single molecular level [1]. Two-photon excitation compared with one-photon and dual-color excitations benefits from deeper penetration, smaller back-scattering, photodamage, and a simple experimental setup for exciting two distinct labels simultaneously. Recently, we have developed the two-photon FCS technique with a time-correlated single-photon counting (TCSPC) system on a Nikon microscope. With this combination, both time-evolution of fluorescence fluctuation and fluorescence decay were recorded to differentiate the contributions of various fluorophores and to eliminate afterpulsing effects [2]. We used 100 nm beads and Rhodamine 6G to calibrate this microsystem and obtained an effective volume of 0.38±0.087 fL ($w_0 = 0.36$ and $z_0 = 1.46 \mu m$).

Two-component systems (2CS), normally composed of a sensor kinase and a response regulator, are important for bacteria to sense environmental stimuli and produce appropriate responses. Certain 2CS possess an additional component called Hpt, which is required for successful phosphorelay signaling. In Pseudomonas aeruginosa, one of the leading gram-negative bacterial pathogens, there are 12 orphan sensor kinases, 3 Hpt proteins and 10 orphan response regulators. These proteins apparently form a complex regulatory network, although how these components interact with each other is not clear. In this work, we investigated the interactions between these proteins by monitoring the changes of diffusion coefficient with TPFCS. In summary, our result supports the possibility of TPFCS to elucidate protein-protein interaction in bacterial signaling pathways.