

**DETERMINING THE HYDRODYNAMIC SIZE AND SHAPE OF  
FLUORESCENT BIOMOLECULES BY PROBING THEIR SINGLE-  
MOLECULE BROWNIAN MOTION**

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Monitoring the Brownian motion of a fluorescent biomolecule in solution renders information regarding its hydrodynamic shape *and* size under physiological conditions. In contrast to the translational diffusion of a fluorescent biomolecule that typically occurs on the micro- to millisecond time scale, its size is more sensitive to its rotational diffusion dynamics occurring on the pico- and nanosecond time scale. While the former is conveniently obtained from a conventional fluorescence correlation spectroscopy experiment, the latter is generally obtained from a time-resolved fluorescence anisotropy experiment upon pulsed excitation, which is inherently limited to the measurement of rotational correlation times not exceeding the fluorescence lifetime of the fluorophore.

To circumvent this problem and to provide an accurate measurement of the rotational diffusion time of a biological macromolecule, which is typically in the order of tens of nanoseconds, we apply a recently developed photon counting technology for the measurement of fluorescence correlation from picoseconds to seconds by registering distinct photon arrival times with picosecond resolution. Utilization of this technique in a polarization-sensitive manner along with an exact theoretical analysis in terms of the second-order correlation function [1] allows us to probe simultaneously the translational *and* rotational diffusion of fluorescent biomolecules. We demonstrate the application of this novel single-molecule methodology for the determination of the hydrodynamic size *and* shape of small dye molecules (with sizes below 10 Å) as well as of fluorescent proteins.

[1] S.R. Aragon and R. Pecora, *Biopolymers*, 14 (1975) 119.