Biased Diffusion, Optical Trapping, and Manipulation of Single Molecules in Solution

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The detection of a single molecule in solution has recently aroused considerable interest, both for its analytical utility and for gaining insight into the chemistry and dynamics at the single-molecule level.[1−3] The ability to manipulate a single molecule in solution has even more exciting possibilities. In this communication, we present our investigation of the diffusional behavior of a single molecule near the focal volume of a confocal fluorescence microscope. We report the first direct optical trapping and manipulation of a single molecule in solution, in particular, a single λ DNA molecule (≈48 kb, isolated from the bacteriophage lambda and purchased from New England Biolabs) labeled with YOYO dye (≈1 YOYO per 20 bases) under conditions in which the λ DNA supercoils. Many studies have been made on DNA in which it is stretched or transported by attaching DNA to a polystyrene bead and using an optical tweezer to exert force on the bead.[4−6] This report uses the same physical principles, but it describes the direct action of the radiation field on DNA.

In confocal fluorescence microscopy, the entrance of a single molecule into the laser probe volume is generally assumed to follow Poissonian statistics which characterizes the time behavior of uncorrelated random events.[7−9] At long timescales, we found complete agreement with the Poissonian model, in which the distribution of intervals of entry into the probe volume decreases exponentially with time. At short timescales, however, we found dramatic deviation from the behavior predicted by Poisson statistics. Figure 1 shows a representative signal from a 14 nm fluorescent polystyrene sphere containing roughly 15 fluorescein-like dye molecules per particle. When the sphere enters the probe volume, it emits a burst of photons that gives rise to a sharp peak in our spectrum. It can be seen from the inset of Figure 1 that several photon bursts are often buried within a larger burst when the timescale is expanded. These buried bursts are most likely caused by the same molecule recrossing into the probe volume. Figure 2 shows the Poissonian analysis of the resulting signal from the 14 nm particles after many spectra were accumulated.[10] The straight line drawn is the least-squares fit to the Poissonian distribution. A large deviation from the fit begins at about 600 ms; the re-entry probability is 18.9 times higher than that expected between 6 and 60 ms where the deviation is most pronounced.

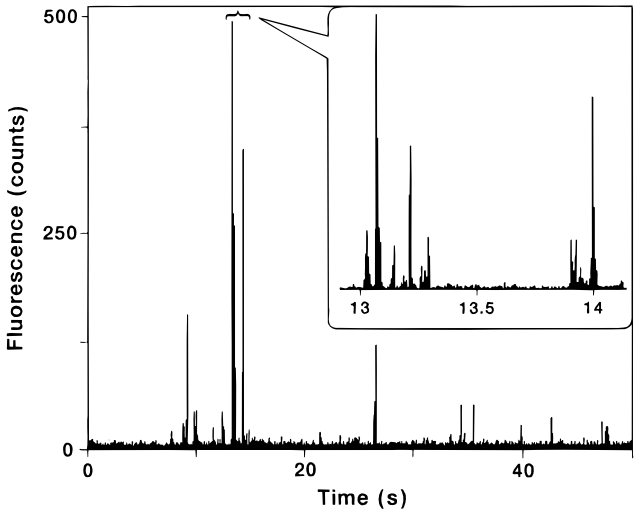

Figure 1. Signal observed from a 10^{-11} M solution of fluorescent polystyrene spheres. Data acquisition speed is 167 points/s (6 ms integration), and the CW laser excitation has a wavelength of 488 nm at a power of 0.5 mW. The inset is an expanded view of the bracketed section of the data.

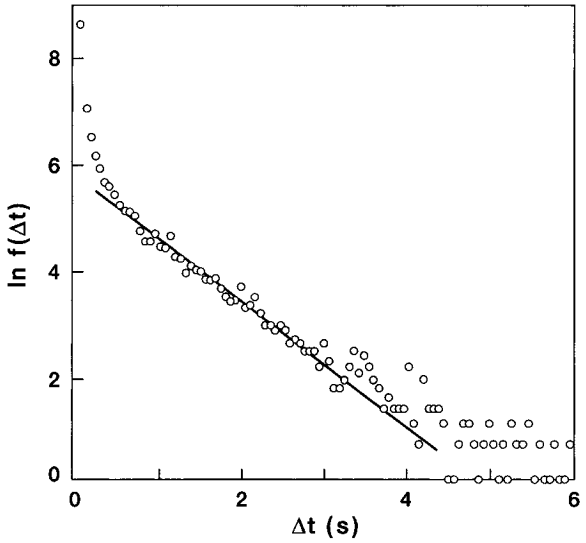

Figure 2. Plot of the logarithm of the photon burst interval distribution $F(\Delta t)$ vs time interval Δt. The straight line represents the best fit to Poisson statistics.

Part of the deviation can be explained by the recrossings of the same molecule into the focal volume once it is already in the vicinity of the probe. This probability is approximately 35% for unbiased diffusion,[9] which is insufficient to explain the actual deviation. In view of this discrepancy, we postulate that the increased recrossing frequencies is caused by an optical trapping potential that arises from the interaction between the electric field of the laser beam and the electric dipole moment induced by the laser beam in the molecule through its polarizability.[11] To test this hypothesis, we superimposed the probe beam (488 nm at 0.5 mW from an argon ion laser) with a trapping beam (830 nm at variable power from a diode laser). This procedure allows us to vary the trapping potential independently without perturbing the signal generated by the probe beam. We find in a typical data set that whereas with no power in the trapping beam the number of recrossings between 6 and 60 ms is 5881, the recrossing number becomes 7407 at 10 mW, 8243 at 17 mW, 9244 at 24 mW, and 10 716 at 31 mW. As expected, the

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(10) The distribution of photon burst intervals that satisfies Poisson statistics is given by $F(\Delta t) = \beta \exp(-\beta \Delta t)$, where β is the characteristic appearance frequency of a photon burst, Δt is the time interval between successive photon bursts, and $F(\Delta t)$ is the number of photon bursts that occur with value Δt. A plot of $\ln(F(\Delta t))$ vs Δt should result in a straight line for photon bursts satisfying the Poisson statistics.

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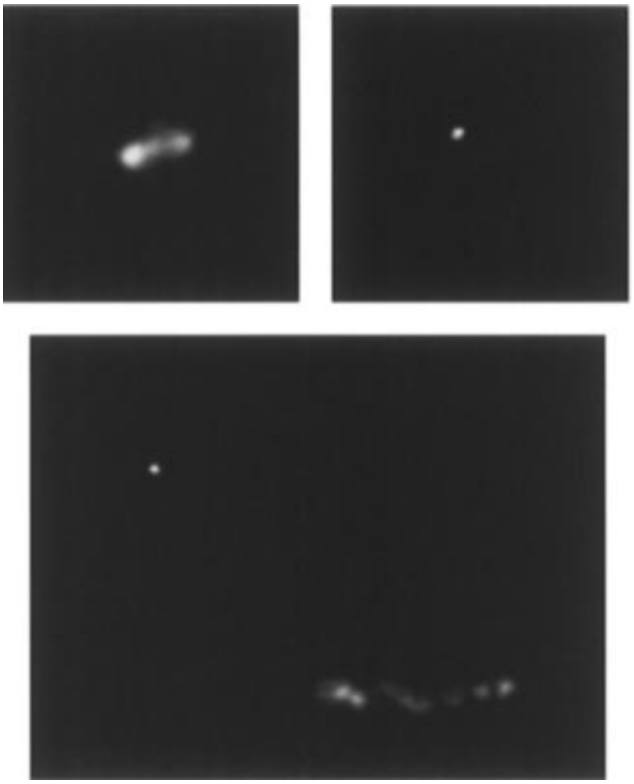

Figure 3. Video image through the microscope of YOYO-intercalated λ DNA in 10 mM Tris, 1 mM EDTA, 2 mM NaCl, and 1% β-mercaptoethanol. The solution also contains 50 μg/mL glucose oxidase, 10 μg/mL catalase, and 0.1% glucose for enzymatic scavanging of oxygen. (a, top left) Image at pH 8 showing extended structure. (b, top right) Image at pH 5.75 showing supercoiled structure. (c, bottom) A 2-s time-averaged image of an optically trapped (upper left corner) and free (lower part) λ DNA at pH 5.75 while the microscope stage is being translated to the left.

number of recrossings increased with increasing trapping power. This behavior indicates that the trapping potential at low laser power, although not sufficiently deep for stable confinement, can significantly bias the diffusional behavior of a single molecule near the focal volume. It is therefore necessary to take into account both the presence of the trapping potential and the unbiased recrossing probability when investigating the behavior and dynamics of a single molecule in and near the focal volume of a confocal fluorescence microscope. We find similar early-time deviations from Poissonian statistics for several fluorescein-labeled proteins in the mass range of 400−600 kDa, including β-galactosidase and alkaline phosphatase which have a physical size roughly between 7 and 10 nm. The extent of the actual deviation varies depending on the particular protein molecule and also on the buffer system and ionic strength.

Indeed, at moderate powers of about 200 mW (at 647 nm) we can easily trap and manipulate a single λ DNA molecule which is in the supercoiled conformation (see Figure 3). The trapping efficiency for intact λ DNA molecules is nearly 100%. At pH 8, as shown in Figure 3a, the λ DNA has a fairly elongated conformation (over a few microns), and it fluctuates rapidly in solution. In this conformation, only a small segment of the DNA is under the influence of the optical trap which is about 0.5 μm in diameter, and the DNA molecule cannot be stably held. At pH 5.75 (2 mM NaCl), as shown in Figure 3b, the λ DNA forms a compact supercoiled structure. The mechanism of this compact DNA formation is not known, but the pH value at which it forms seems to correlate with the formation of H-DNA.[12] In this supercoiled conformation, the λ DNA is readily trapped and moved at will. The upper left corner of Figure 3c shows the fluorescent image (time averaged for 2 s) of a trapped DNA while the microscope stage is translated to the left. The trapped DNA remains clearly defined and is not blurred in time. The light trail at the bottom of the picture is caused by fluorescence from a free DNA while the stage is moved. The ability to trap a single DNA molecule in solution opens exciting possibilities not only for manipulation purposes but also as a tool to study DNA conformational changes. We are currently investigating the dynamics of supercoiled DNA formation by first trapping the DNA, followed by introduction of a base to raise the pH. When the trap is subsequently turned off, the DNA should uncoil in this higher pH environment. This technique enables us to study directly at the single-molecule level the supercoiling and uncoiling of DNA. In addition to pH, the formation of supercoiled DNA is also strongly dependent on the amount of intercalated dye molecules. The ability to hold in solution a single molecule, albeit at present a fairly large one, and to position it at will represents an important advance in nanoscale chemical control.

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