

# Study of Quantitative and Dynamics Polymer Particle Tracking

B Ramesh

Research Scholar, CMJ University, Shillong, Meghalaya

**Abstract:** *We present a new method for analyzing the dynamics of conformational fluctuations of individual flexible polymer molecules. In single-particle tracking (SPT), one end of the polymer molecule is tethered to an immobile substratum. A microsphere attached to the other end serves as an optical marker. The conformational fluctuations of the polymer molecule can be measured by optical microscopy via the motion of the microsphere. The bead-and-spring theory for polymer dynamics is further developed to account for the microsphere, and together the measurement and the theory yield quantitative information about molecular conformations and dynamics under nonperturbing conditions. Applying the method to measurements carried out on DNA molecules provides information complementary to recent studies of single DNA molecules under extensional force. Combining high precision measurements with the theoretical analysis presented here creates a powerful tool for studying conformational dynamics of biological and synthetic macromolecules at the single-molecule level.*

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## INTRODUCTION

The theory of polymer dynamics is one of the most elegant and cogent subjects in macromolecular science (Doi and Edwards, 1986). The fundamental difference between the physics and chemistry of a small molecule and that of a macromolecule is that the latter can adopt a vast number of approximately equally probable conformations. Any individual polymer molecule within a population constantly fluctuates among these conformations; the populations must be characterized and analyzed in terms of a distribution. In the past, almost all experimental measurements on polymers, either thermodynamic or hydrodynamic, yielded averaged behavior of the whole population distribution (Flory, 1969; Doi and Edwards, 1986). These measurements have validated the statistical theory of polymer conformations and have provided much important information about a wide range of both synthetic and biological polymers. They do not directly reveal, however, the conformational dynamics of individual polymer molecules. For biological polymers like DNA the rates of conformational fluctuations can be of great significance in biochemical processes such as the binding of Lac repressor protein to its operon for gene expression (Finzi and Gelles, 1995; Shore et al., 1981; Horowitz and Wang, 1984).

With the technological advancement in micromanipulation and video-enhanced optical microscopy, one now can study single DNA molecules in aqueous solution (Smith et al., 1992). Large spontaneous dynamic fluctuations of individual macromolecules can be observed by optical microscopy. In a typical experiment, one end of the polymer

molecule is anchored to a substratum. A microsphere bound to the other end as an optical marker is tracked using video-enhanced optical microscopy with a nanometer precision (Gelles et al., 1988). This approach has recently been applied to measure the kinetics of DNA looping induced by Lac repressor protein. The spatial range of the spontaneous conformation fluctuations of the tethered DNA molecule indicated whether its effective length had been shortened by the formation of an interior loop (Finzi and Gelles, 1995). Although there was no attempt to study the intrinsic properties of the DNA molecule itself in these experiments, this method of tethered particle motion (TPM) (Yin et al., 1994) can also yield much information about the equilibrium and dynamics of conformational fluctuations of the tethering DNA molecule and of other flexible polymer tethers. The method belongs to a class of optical methods based on particle tracking (Gelles et al., 1988; Qian et al., 1991; Mason et al., 1997; Gittes et al., 1997). In this paper we propose the use of single-particle tracking (SPT) to study polymer conformational dynamics at the level of the individual molecule. This novel approach provides direct observations of conformational fluctuations of individual macromolecules under relatively nonperturbing conditions, complementary to recent studies of single DNA molecules under extensional force (Smith et al., 1992, 1996; Perkins et al., 1994, 1995; Cluzel et al., 1996; Strick et al., 1996).

The fundamental difference between traditional macroscopic studies and the recent single-molecule measurements is that the thermal fluctuations are an intrinsic and essential contribution rather than an undesirable source of noise (Elson and Webb, 1975;

Elson and Qian, 1994; Shapiro and Qian, 1997, 1998), reminiscent of the pioneering studies on single channel proteins in membranes that have revolutionized physiology (Neher and Stevens, 1977). In this paper we develop a quantitative basis for interpreting measurements of the stochastic behavior of single macromolecular tethers (e.g., DNA) based on the theory of polymer dynamics (Rouse, 1953; Zimm, 1956; Doi and Edwards, 1986). We anticipate that the quantitative analysis of fluctuating polymers will become a powerful tool for studying the relationship between structures and dynamical properties of flexible polymers as well as biological macromolecules.

Measurements of the transient relaxation of a linear thermodynamic system after a perturbation yield information identical to that obtained from measurements of spontaneous fluctuations (Keizer, 1987). For a nonlinear system, e.g., a random coil polymer at large extension, the stretching experiments are dominated by the effects of the nonlinearity. The fluctuation measurements, however, probe the linear elasticity of the system. A fluctuation measurement requires many measurements over time (i.e., the measurements of many fluctuations) to achieve statistical accuracy. The time resolution of the kinetics is not determined, however, by the duration of the measurement. A longer measurement time yields a higher accuracy, by averaging over both random measurement errors and variations due to the stochastic character of the spontaneous fluctuations. Similarly, averaging over many transients in a relaxation experiment improves the accuracy. Finally, a measurement in which a single polymer is extended by external force selectively probes the modes with fast relaxation and less thermal fluctuations, whereas an equilibrium fluctuation measurement probes the modes equally with fast and slow relaxations (equipartition).

To apply the theory of polymer dynamics to SPT, one has to take explicit account of the microsphere (optical marker). This requires a reformulation of the established theory (Doi and Edwards, 1986) and leads to a different mathematical problem with a transcendental eigen equation. To clearly illustrate the theoretical development, we present analyses for both free-draining polymer (Rouse, 1953) and polymer with hydrodynamic interaction (Zimm, 1956). The former is conceptually simple but applicable only to short polymer molecules, whereas the latter is more appropriate for long polymers. The paper is organized as follows. In the next section we describe a simple quantitative method for analyzing conformational fluctuations of flexible macromolecules such as DNA. The third section presents an analysis of the dependence of the observed polymer dynamics on the presence of the tethered particle and the length of the tether. The fourth section provides an example of the application of the

analytical method to experimental measurements. The last section discusses the significance of this approach to the study of biochemical processes and polymer dynamics.

## ANALYSIS OF CONFORMATIONAL FLUCTUATIONS

In SPT measurements the position of the microsphere, tethered by a flexible polymer molecule to the substratum, fluctuates over time. The microsphere position can be simply defined as the center of its optical image. Its distance to the site of anchorage to the substratum defines the end-to-end distance (Flory, 1969; Doi and Edwards, 1986) of the tether. A histogram and a time correlation function (Elson and Webb, 1975; Qian et al., 1991) of the microsphere position respectively characterize statistically the equilibrium and the dynamic properties of the tether (Fig. 1). Our first task is to interpret the measured fluctuations of the position of the tethered particle in terms of the structural and dynamic properties of the polymer molecule. The simplest analysis is based on the phenomenological dumbbell model for polymer dynamics (Bird et al., 1987), according to which the particle is assumed to be tethered to the substratum by a simple linear spring with effective spring constant  $k_{db}$ . This spring constant is determined both by the flexibility and by the length of the polymer tether, as discussed below. The viscous resistance to the motion of the microsphere defines an overall relaxation time constant ( $\tau_1$ ). The time constant characterizes the rate of motion of the harmonically bound particle in its viscous environment.

The equilibrium distribution of the positions of the microsphere should be independent of both its frictional coefficient and size if we neglect its excluded volume effect with both the substratum and the tether itself. Therefore, under these assumptions the SPT measurements should faithfully yield the distribution of polymer end-to-end distances ( $r$ ). The dumbbell model characterizes the dynamics

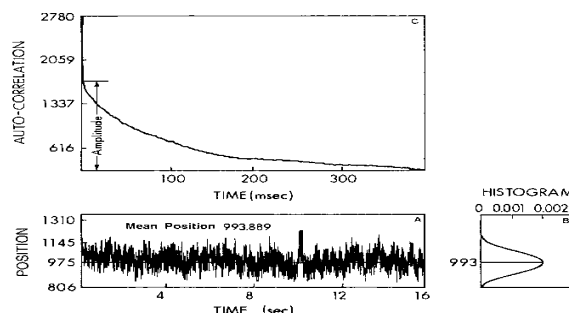


FIGURE 1 A schematic illustration of the analysis of the fluctuating position of the optical marker, in terms of a distribution or histogram and an autocorrelation function of the position measurements. The position fluctuation data

are in arbitrary length units. The distribution gives the mean and variance of the fluctuating position. The autocorrelation function yields a correlation time ( $\tau_1$ ) and its amplitude is the same as the variance. For a sequence of data  $\{x_n\}_{n=1, 2, \dots, N}$ , the autocorrelation function is obtained as

$$g(m) = \frac{1}{N-m} \sum_{n=1}^{N-m} x_n x_{n+m} - \left( \frac{1}{N} \sum_{n=1}^N x_n \right)^2$$

where  $m$  usually is much smaller than  $N$ .

of the polymer tether in terms of a (Langevin) equation of motion (Bird et al., 1987) for the end-to-end vector  $\mathbf{r}$  in a viscous solvent:

$$\zeta_{db} \frac{d\mathbf{r}}{dt} = -k_{db} \mathbf{r} + \mathbf{f}_{db}(t) \quad (1)$$

where we neglect the inertial term and  $\mathbf{f}_{db}(t)$  is a fluctuating force due to the thermal motions of the solvent molecules. The subscript  $db$  specifies the dumbbell model. In effect, the stochastic differential equation (Eq. 1) describes the diffusion of the microsphere in the harmonic potential to which it is bound and so is mathematically equivalent to a partial differential equation in the form of a diffusion equation for the probability distribution  $P(\mathbf{r}, t) = P(x, t)P(y, t)P(z, t)$  (Wax, 1954; Van Kampen, 1992):

$$\frac{\partial P(x, t)}{\partial t} = D \frac{\partial^2 P(x, t)}{\partial x^2} + \frac{\partial}{\partial x} \left[ \frac{k_{db}}{\zeta_{db}} x P(x, t) \right] \quad (2)$$

where  $2\zeta_{db}D\delta(\tau) = \langle \mathbf{f}_{db}(t)\mathbf{f}_{db}(t + \tau) \rangle$ ;  $D$  is a diffusion coefficient; and  $\langle \cdot \rangle$  denotes an ensemble average. The equilibrium solution of Eq. 2 is a Gaussian distribution ( $P_{eq}(\mathbf{r})$ ) for the end-to-end distance  $r = |\mathbf{r}|$  with variance  $3D\zeta_{db}/k_{db}$ . Thus far the relationship between the stochastic differential equation (Eq. 1) and the partial differential equation (Eq. 2) is strictly mathematical (Van Kampen, 1992). We can further determine the relationship between  $\zeta_{db}$  and  $D$  from thermal physics. At thermal equilibrium, the mean square end-to-end distance, i.e., the variance of the distribution of end-to-end distances, is  $3k_B T/k_{db}$ , where  $k_B$  is Boltzmann's constant and  $T$  is the temperature in Kelvins (Wax, 1954; Van Kampen, 1992). Hence we have  $D = k_B T/\zeta_{db}$ , which is the well-known Einstein formula, a special case of the general fluctuation-dissipation relation for equilibrium thermal fluctuations (Keizer, 1987; Klapper and Qian, 1998). Therefore, the equilibrium distribution for the end-to-end distance  $r$  is a simple Boltzmann distribution:

$$P_{eq}(\mathbf{r}) = P_{eq}(x, y, z) \propto e^{-k_{db}(x^2 + y^2 + z^2)/2k_B T}$$

and

$$P_{eq}(r) \propto r^2 e^{-k_{db}r^2/2k_B T} \quad (3)$$

For the microsphere diffusing in the harmonic potential well, we can determine analytically from Eq. 2 not only the equilibrium distribution  $P_{eq}(\mathbf{r})$  but also the temporal behavior from the fundamental solution (Green's function)  $P(\mathbf{r}, t)$ ,

which yields the probability that, if the microsphere is at position  $\mathbf{r}'$  at some reference time, it is at position  $\mathbf{r}$  after an elapsed time interval  $t$ . The measured time-dependent fluctuations in  $r$  are most readily characterized statistically, in terms of the time correlation function (Elson and Webb, 1975; Qian et al., 1991):

$$G(t) = \frac{\int \int \mathbf{r} \mathbf{r}' P(\mathbf{r}, t | \mathbf{r}') P_{eq}(\mathbf{r}') d\mathbf{r} d\mathbf{r}' - \int \mathbf{r}^2 P_{eq}(\mathbf{r}) d\mathbf{r}}{\int \mathbf{r}^2 P_{eq}(\mathbf{r}) d\mathbf{r}} \quad (4)$$

where  $\tau_1 = \zeta_{db}/k_{db}$ , the conformational relaxation time of the tethering polymer molecule. At any moment  $r$  is likely to deviate from its equilibrium value. The time required to relax to the equilibrium value is characterized by  $\tau_1$ , which depends on the elasticity ( $k_{db}$ ) of the polymer segment and the overall viscous resistance to motion ( $\zeta_{db}$ ) of the polymer and the microsphere through the solvent. From the time record of the measured coordinates of the microsphere, the correlation function in Eq. 4 can be computed to yield the relaxation time  $\tau_1$  (see more details in Fig. 1).

For a weakly bending polymer molecule, the length over which it can substantially bend is called a persistence length ( $\ell_p$ ). Molecules for which the contour length is much greater than the persistence length can be considered as random coils. The dumbbell model can be applied to microspheres tethered either by stiff polymers with less than one persistence length or by random coils with many persistence lengths, but the energetics of the elastic restoring force are different for the two types of tethers. Contributing to the force constant  $k_{db}$  in Eq. 3 are both entropic forces originating from the distribution of the polymer over its random coil conformations and enthalpic forces that resist bending over a range of distances that are small compared to the polymer persistence or segment length. Hence there should be contributions to  $k_{db}$  that are both proportional to and independent of temperature  $T$ :

$$k_{db}(T) = a + bT. \quad (5)$$

For a polymer with many persistence lengths (elastic dumbbell),  $k_{db} \approx 3k_B T / 2L\ell_p$ , where  $L$  is the contour length of the polymer (Flory, 1969). Hence,  $k_{db}$  varies inversely with the length of the tether,  $L$ , i.e., as  $1/L$ . If, however, the polymer is short (rigid dumbbell, less than one persistence length), then the backbone bending (the  $a$  term) dominates  $k_{db}$ . For a stiff tether the  $k_{db}$  due to transverse bending varies as  $1/L^3$  (Barkley and Zimm, 1979). The temperature dependence of  $k_{db}$  (i.e., Eq. 5) provides an informative indicator of the source of the flexibility and elasticity of the tethering polymer, and a way to distinguish whether the molecule is behaving as a flexible random coil or a weakly bending beam and whether the bending is static or dynamic (Schellman and Harvey, 1995). Helical DNA behaves as a weakly bending beam for lengths shorter than 150 bp and as a wormlike random coil for much greater lengths. Hence, in SPT experiments on DNA, it is useful to carry out measurements of conformational dynamics as a function of temperature to characterize the energetics of the elastic restoring force and to determine the nature of the conformational fluctuations being observed.

## DISCUSSION

To illustrate the principles underlying dynamic SPT measurements, we have discussed only the simplest models for macromolecular conformations and dynamics. Even at this level, it is clear that much interesting information about the dynamics of motion of the tether can be obtained using this approach. More realistic models would consider the excluded volume and hydrodynamic interactions of the tethered particle and the polymer segments, the detailed relationship between the structure and dynamic properties of the tether, the influence of the substratum, which limits the polymer conformations, and time averaging effects on the actual measurements. These subjects will be carefully investigated in future work.

Let us consider whether the time resolution of present SPT technology is sufficient to study the conformational dynamics of single DNA molecules. Analysis of dynamic light scattering measurements on DNA in terms of Rouse-Zimm theory has yielded the frictional coefficient  $\zeta \approx 10^{-9}$  N s/m and spring constant  $k \approx 3 \times 10^{-7}$  N/m (Lin and Schurr, 1978). On the other hand, according to Stokes' law, the frictional coefficient ( $\zeta'$ ) for a spherical particle with diameter  $d$  is  $3\pi\eta d$ , where the viscosity of water  $\eta = 10^{-3}$  N s/m<sup>2</sup>. Hence for  $d = 0.23$   $\mu$ m, we have  $\zeta' \approx 2.27 \times 10^{-9}$  N s/m in water. This leads to  $\zeta/\zeta' \approx 0.5$  and a relaxation time ( $\tau_1$  in Eq. 12) for a 1000-bp DNA molecule of  $\sim 120$  ms,

which is within the reach of the time resolution of video microscopy (33 ms). Of course, a longer DNA molecule will yield a larger  $\tau_1$  (cf. Eq. 12), which could be determined more precisely under the same time resolution.

In recent years, it has become increasingly clear that, in addition to carrying genetic information in its sequence, the structure and dynamics of DNA also play central roles in the biochemical processes of decoding the information, processes such as the initiation and regulation of gene transcription. Hence, applying quantitative measurements such as SPT to DNA with specific sequences will greatly enhance our understanding of DNA conformational fluctuation effects on these important regulatory processes. For example, there is ongoing search on the nature of "action at a distance" (Wang and Giaevers, 1988; Rippe et al., 1995). One of the possible mechanisms for this general phenomenon is that flexible DNA molecules form loops stabilized by specifically bound protein molecules, i.e., binding-induced bending. The average probability of DNA loop formation has been experimentally measured in terms of cyclization (Shore et al., 1981; Horowitz and Wang, 1984). The method developed here will provide further information on individual DNA molecules, which range in behavior from weak bending to flexible circles. Applying these quantitative measurements to DNA with specific sequences will greatly enhance our understanding of various important biochemical processes.

The method developed here is not limited to studying DNA polymers. It can be applied to other synthetic and biological polymers or even polymer networks. Combining delicate experimental measurements and theoretical analyses, this new SPT method can provide important information on the molecular mechanics of diverse biological macromolecules. For example, consider the physiologically important unfolding of the giant muscle protein, titin, in response to extensional force in three recent studies based on force microscopy and optical trapping of single protein molecules (Reif et al., 1997; Kellermayer et al., 1997; Tskhovrebova et al., 1997). Titin provides a restoring force that resists excessive extension of the muscle. The molecule behaves as a complex spring in which successive unfolding of various types of conformational domains with different characteristics provides resistance to stretch as well as compliance (Erickson, 1994). These studies have revealed a wide range of interesting behaviors of this protein but also indicate significant effects of the stiffness of the probes and the rate of application of force to the molecule. These effects complicate the interpretation of the measurements (Shapiro and Qian, 1997, 1998). Measurement of the fluctuating conformation of the protein under nonperturbative



conditions using SPT yields molecular characteristics in a well-defined, zero-load state and provides complementary information on this molecule. Specifically, the relative contributions of the wormlike chain elasticity and cooperative protein domain folding-unfolding transition in the native state of the protein can be studied (Bustamante et al., 1994). The relative time scales of these two processes could be obtained if one chose appropriate temperature or solvent conditions to set the protein at the middle of the unfolding transition of a specific domain.

Measurements of the transient response of single macromolecules under external force is now a well-established biophysical technique. These measurements involve large thermal fluctuations due to Brownian motion on the single-molecule level. With the SPT method, we take advantage of these large fluctuations and from them obtain valuable dynamic information on a relatively unperturbed state of the molecule, as was first suggested by Einstein almost a century ago (Einstein, 1956).

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