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Citation: The Journal of Chemical Physics **117**, 6372 (2002); doi: 10.1063/1.1503335
View online: http://dx.doi.org/10.1063/1.1503335
View Table of Contents: http://scitation.aip.org/content/aip/journal/jcp/117/13?ver=pdfcov
Published by the AIP Publishing

Sample size autocorrelation analysis of kinetic data: Resolving reaction path heterogeneity

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(Received 12 April 2002; accepted 8 July 2002)

Detailed theory on a method of analysis of kinetic data based on sample size autocorrelation function was developed to identify the presence of reaction path heterogeneity based on the master equation approach. The behavior of such functions under ideal conditions as well as under heterogeneity conditions was discussed and some of the potential applications of this method have been summarized. Application of this theory to renaturation kinetics of DNA not only revealed the presence of path heterogeneity in the second zipping phase and quantified it but also proved that it was due to nonreactive modes of dynamics on conformational energy landscape, which agreed well with earlier studies. © *2002 American Institute of Physics.* [DOI: 10.1063/1.1503335]

INTRODUCTION

Analysis and interpretation of kinetic data is crucial from ordinary chemical reactions involving simple molecules to biological reactions involving complex macromolecules. In the reactions involving simple molecules the so-called reaction path heterogeneity will be negligible since most of the molecules follow a single path between initial and final states (generally it can be described by two-dimensional energy diagrams where we keep free energy as ordinate and the reaction coordinate as abscissa). But is not easy to describe the kinetics of macromolecules by such kind of simple two-dimensional diagrams. Here we use generally the energy landscape-funnels. The theory of such landscapes has already been well developed and applied in problems such as protein folding, etc.[1–5] In such cases, normal analysis of kinetic data will give only the estimate of average rate constants and thus we cannot get any information regarding the path heterogeneity. The rate constants obtained from aforementioned systems have meaning only when the landscape funnel is symmetric and consists of only two levels without any significant kinetic traps as they lead to bifurcation of paths with different time scales. The usual kinetic analysis cannot resolve the bifurcation phenomenon. A lot of work has already been done to identify and follow the reaction paths.[6,7] But there was no general and experimentally applicable method to identify and quantify the reaction path heterogeneity itself just from simple kinetic data. Since this kind of analysis has potential applications, especially in the studies involving conformational transitions of biopolymers like protein and DNA in the presence as well as the absence of molecular chaperones, there is a need to develop a sensitive method of analysis to interpret the kinetic data. In this article I present a simple tool called as ''sample autocorrelation analysis'' to analyze kinetic data for the presence of reaction path heterogeneity and also to quantify it. First, the formal theory will be developed and subsequently some of its applications in biological systems will be presented.

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AUTOCORRELATION FUNCTION

Autocorrelation function that was first described by Taylor[8] can be defined as

$$G(\tau)=\lim_{T\to\infty}\frac{1}{T}\int_0^T g(t)g(t+\tau)dt. \tag{1}$$

Equation (1) will hold only for the continuous data points. Since usually the experimental data points are discrete, the following equation can be used to calculate the normalized autocorrelation function:

$$G(\tau)=\frac{\langle g_i,g_{i+\tau}\rangle}{[\langle g_i,g_i\rangle\langle g_{i+\tau},g_{i+\tau}\rangle]^{0.5}}=\frac{\sum_{i=0}^{N-\tau}g_ig_{i+\tau}-\left(\frac{\sum_{i=0}^{N-\tau}g_i\sum_{i=0}^{N-\tau}g_{i+\tau}}{N-\tau}\right)}{\sigma_{g_i}\sigma_{g_{i+\tau}}}, \tag{2}$$

where

$$\sigma^2_{g_{i+\tau}}=\sum_{i=\tau}^{N-\tau}g^2_{i=\tau}-\frac{\left(\sum_{i=0}^{N-\tau}g_{i+\tau}\right)}{N-\tau},$$

$$\sigma^2_{g_i}=\sum_{i=0}^{N-\tau}g_i^2-\frac{\left(\sum_{i=0}^{N-\tau}g_i\right)^2}{N-\tau}.$$

Here N is the total number of data points available, τ is the autocorrelation delay, $N\neq\tau$, $\tau<N$, $G(\tau)=G(-\tau)$, and $-1\leqslant G(\tau)\leqslant 1$. The bracket notation in Eq. (2) denotes averaging with time. The commercial autocorrelators will generally keep N as a constant (to its maximum) and construct $G(\tau)$ by slowly increasing the τ value.

SAMPLE SIZE AUTOCORRELATION

From the last paragraph it is clear that autocorrelation is a function of ''autocorrelation delay'' τ. Suppose let us take

only first n data points out of N to calculate autocorrelation as given by Eq. (2) and call it "sample size autocorrelation $G(\tau,n)$" due to the fact that it is also a function of sample size n. We can deduce the mathematical properties of such function as follows: Any kinetic data (e.g., time evolution of a spectroscopic variable) contain two major components. One is from the deterministic functional dependency (for an ideal situation it is just the solution of deterministic differential equation) and the other one is noise,

$$S(t)=f(t)+\xi(t). \tag{3}$$

Here, $S(t)$ is the observed value, $f(t)$ is the functional dependence (due to only reactive modes and $d_t f\neq 0$), and $\xi(t)$ is noise at time t. Here the so-called noise may enter in two ways, namely from the instrument $[e(t)]$ and from the reactive system itself $[h(t)]$. Therefore we can encounter the following two cases.

Case I: $\xi(t)=e(t)$: $\xi(t)=e(t)$ occurs when the system under study is a simple [i.e., reactions that can be described by two-dimensional energy diagrams or with unconnected (probabilistic) conformational domains of reactant] one. We assume that $e(t)$ is an additive Gaussian noise with[9] $\langle e\rangle=0$ and $\sigma_e^2\approx 1/n$ for $n\geqslant 30$ (here n is sample size), $\langle e_i,e_{i+\tau}\rangle=0,\langle e,f\rangle=0$. The normalized sample size autocorrelation function corresponding to $S(t)=f(t)+e(t)$ can be written as

$$G(\tau,n)=\frac{\langle S_i,S_{i+\tau}\rangle}{[\langle S_i,S_i\rangle\langle S_{i+\tau},S_{i+\tau}\rangle]^{0.5}}=\frac{\langle f_i+e_i,f_{i+\tau}+e_{i+\tau}\rangle}{[\langle f_i+e_i,f_i+e_i\rangle\langle f_{i+\tau}+e_{i+\tau},f_{i+\tau}+e_{i+\tau}\rangle]^{0.5}}. \tag{4}$$

Expanding the terms present in the brackets in Eq. (4),

$$G(\tau,n)=\frac{\langle f_i,f_{i+\tau}\rangle+\langle e_i,e_{i+\tau}\rangle+\langle f_i e_{i+\tau}\rangle+\langle e_i,f_{i+\tau}\rangle}{[(\langle f_i,f_i\rangle+\langle e_i,e_i\rangle+2\langle f_i,e_i\rangle)\times(\langle f_{i+\tau},f_{i+\tau}\rangle+\langle e_{i+\tau},e_{i\tau}\rangle+2\langle f_{i+\tau},e_{i+\tau}\rangle)]^{0.5}}, \tag{5}$$

and since e and f are uncorrelated functions, Eq. (5) simplifies to

$$G(\tau,n)=\frac{\langle f_i,f_{i+\tau}\rangle}{[(\langle f_i,f_i\rangle+\langle e_i,e_i\rangle)\times(\langle f_{i+\tau},f_{i+\tau}\rangle+\langle e_{i+\tau},e_{i+\tau}\rangle)]^{0.5}}=\frac{\langle f_i,f_{i+\tau}\rangle}{\left[\left(\langle f_i,f_i\rangle+\frac{1}{n}\right)\times\left(\langle f_{i+\tau},f_{i+\tau}\rangle+\frac{1}{n}\right)\right]^{0.5}}, \tag{6}$$

$$\lim_{\tau\to 0} G(\tau,n)=G(\tau_0,n)=\frac{\langle f,f\rangle_{\tau\to 0}}{\langle f,f\rangle_{\tau\to 0}+\frac{1}{n}}. \tag{7}$$

And, it is easy to verify that

$$\lim_{n\to\infty} G(\tau_0,n)=G(\tau_0,n)^{\infty}=1. \tag{8}$$

Thus from Eqs. (7) and (8) we can conclude that if there is only additive instrumental noise apart from reactive modes or if conformational domains of reactants are not connected, then the sample size autocorrelation of the kinetic data will asymptotically attain unity with n.

Case II: $\xi(t)=e(t)+h(t)$: Equation (7) holds only for an ideal situation where the degrees of freedom of the nonreactive mode of dynamics is zero, which is certainly not true in the case of macromolecules where the (probabilistic) conformational domains of reactants are strongly coupled by nonreactive modes. This can be proved as follows: Since the nonreactive mode of dynamics can be well described as a two-dimensional random walk under zero potential, the corresponding Fokker–Planck equation for the probability distribution can be given as

$$\partial_t P(x,y,t)=D(\partial_x^2 P(x,y,t)+\partial_y^2 P(x,y,t)), \tag{9}$$

where D is the internal diffusion coefficient and this can be written in polar coordinates (taking only the radial terms) as,

$$\partial_t P(r,t)=D\left(\partial_r^2 P(r,t)+\frac{1}{r}\partial_r P(r,t)\right). \tag{10}$$

The formal solution to Eq. (10) with initial condition as $P(0,0)=1$ (by the method of separation of variables) can be given as

$$P(r,t)=J_0(r)e^{-Dt}. \tag{11}$$

Since $0\leqslant P(r,t)\leqslant 1$, Eq. (11) is valid only up to first zero of the Bessel's function $J_0(r)$. Therefore the range of r can be given as, $0\leqslant r\leqslant r_0^1$, where $J_0(r_0^1)=(0)_1$. Suppose if the initial position of the ith conformation is r_i, then Eq. (11) can be rewritten as [here $r_i\leqslant r\leqslant(r_0^1+r_i)$],

$$P_i(r,t)=J_0(r_i-r)e^{-Dt}. \tag{12}$$

When r is sufficiently large Eq. (10) can be approximated as follows:

$$\partial_t P(r,t)=D(\partial_r^2 P(r,t)). \tag{13}$$

This is a well-known radial-diffusion equation, which has solution in terms of Gaussian functions (with mean $=r_0'$ and variance $=2Dt$) as

$$P_i(r,t)=\left(\frac{1}{2\sqrt{\pi D(t-t_0)}}\right)e^{[(r-r_0')^2/4D(t-t_0)]}. \tag{14}$$

According to the theory of normal distribution, $\int_{r_0^i}^{r=12Dt} P_i(r,t)dr\approx 0.99$ and thus we can say that two configurational domains j and i at time $t=t_0$ are practically not connected if $|r_0^i-r_0^j|\geqslant 12Dt_0$. But even though the aforementioned condition is true and the domains are not connected at $t=t_0$, since the variance $=2Dt$ is a function of

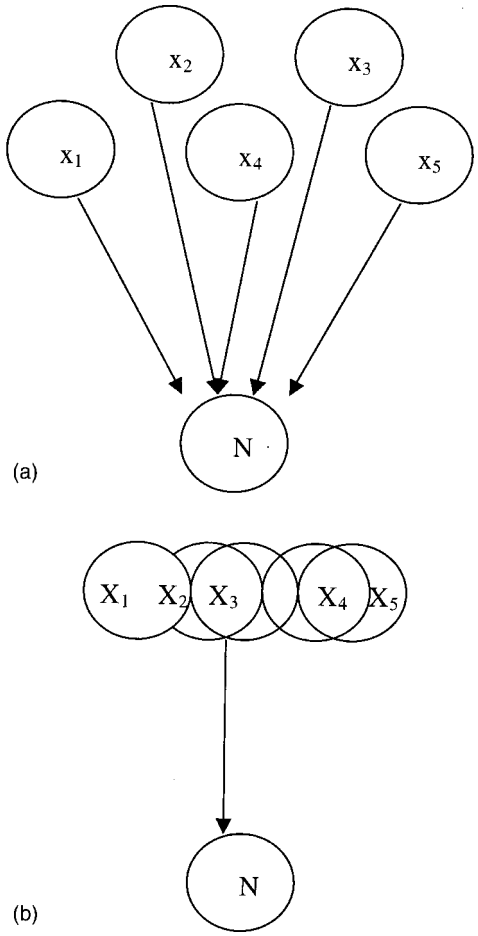

FIG. 1. (a) Here $X_1 \cdots X_5$ are the conformations evolving independently towards N (therefore the path is heterogeneous), which is the end product (in the renaturation problem it is the duplex DNA) due to the fact that their domains are not connected. This also indirectly indicates that conformational fluctuation or lateral diffusion is absent (ideal cases where $\lambda=0$). Here the circles schematically indicate the probabilistic domains. (b) Here $X_1 \cdots X_5$ are the conformations evolving jointly towards N (therefore the path is homogeneous) due to the fact that their domains are connected. This also indirectly indicates the presence of conformational fluctuations or lateral diffusion. Here the circles schematically indicate the probabilistic domains.

time, after certain time t_r of evolution, the inequality will be reversed ($|r_0^i - r_0^j| \leqslant 12Dt_r$) and thus domains j and i will be connected. Thus nearly all the subdomains of configurational space are not only connected but also overlapping. The overlapping area gives the transition probability between these two configurations,

$$\lambda_{ij} = \int_{r_e}^{\infty} (P_i(r,t) + P_j(r,t))dr, \tag{15}$$

where $r_e(r_0^i + r_0^j)/2$ and $0 \leqslant \lambda_{ij} \leqslant 1$.

It is very easy to verify that $\lambda_{ij} = \lambda_{ji}$, which satisfies the condition of detailed balance! Generally reaction path heterogeneity arises due to lateral diffusion on the potential energy landscape. If this lateral diffusivity is very high (i.e., if conformations are in rapid equilibration), the heterogeneity in the reaction path will disappear (therefore path heterogeneity and lateral diffusivity are negatively correlated!). This is due to the fact that strongly connected conformational domains evolve almost coherently with time, which is depicted schematically in Fig. 1(b). Therefore the presence of the reaction path heterogeneity can be indirectly checked by the estimation of fluctuations due to nonreactive models. If these fluctuations are high, then we can conclude that path heterogeneity is low and vice versa. In this case, Eq. (3) becomes as follows:

$$S(t) = f(t) + h(t) + e(t), \tag{16}$$

where $h(t)$ is an additive fluctuation component due to nonreactive modes of conformational transitions. In this article we consider only simple cases, where the conformational transition probabilities are constants. The mean and variance of $h(t)$ of such systems can be calculated as follows: Let there be M number of molecular conformations initially at a given energy level and the transition probabilities from $(x-1)$th conformation to xth conformation in an infinitesimal time Δt, assuming equal initial probability, can be given as follows:

$$\begin{aligned} &\text{Prob}(x-1) \rightarrow x,t) = \lambda DT, \\ &\text{Prob}(x \rightarrow, x, t) = 1 - \lambda \Delta T. \end{aligned} \tag{17}$$

The birth–death master equation corresponding to system (17) becomes as,

$$\partial_t P(x,t) = \lambda P(x-1,t) - \lambda P(x,t). \tag{18}$$

Equation (18) can be simply solved by the method of generating functions[10] as follows:

$$\text{Let,} \quad G(s,t) = \sum_{x=0}^{M} s^x P(x,t). \tag{19}$$

Putting Eq. (19) in Eq. (18), and using $G(s,0) = (1/M)\Sigma_{x=0}^{M} s^x$ as due to the fact that

$$P(x,0) = \frac{1}{M}, \quad \text{for} \quad 0 \leqslant x \leqslant M,$$

$$\partial_t G(s,t) = \lambda(s-1)G(s,t), \tag{20}$$

$$G(s,t) = e^{\lambda(s-1)t}\left(\frac{1}{M}\sum_{x=0}^{M} s^x\right). \tag{21}$$

Then the conformational probability distribution becomes as

$$P(x,t) = \frac{e^{-\lambda t}}{M}\left(\sum_{i=0}^{x} \frac{[\lambda t]^i}{i!}\right). \tag{22}$$

Equation (22) clearly shows that the probability of finding a conformation sufficiently away from the initial position is not effected (independent of time!), i.e., $\lim_{x\to\infty} P(x,t) = 1/M$ and this also indicates a lateral diffusion on the configurational energy landscape. Here the magnitude of diffusivity is solely depending on the parameter λ, which can be interpreted as the connectedness of configurational domains. Since λ and reaction path heterogeneity are negatively correlated (i.e., *path−heterogeneity* $\propto 1/\lambda$), if $1 \ll \lambda$, due to rapid diffusion (or equilibrium), reaction path heterogeneity will disappear (i.e., all the conformations evolve almost identically with time) and for $1 \gg \lambda$, the path becomes fully heterogeneous and tend to even bifurcate (i.e., conformational do-

mains are weakly coupled and thus evolve independently). One also should note that $\lim_{t\to\infty} P(x,t)=0$ as in the case of Langevin free particle and the time evolution of $\langle x(t)\rangle$ and its variance can be given as

$$\langle x(t)\rangle = \lim_{s\to 1} \partial_s G(s,t) = \frac{(M+1)}{2} + \lambda t, \tag{23}$$

$$\begin{aligned}\mathrm{Var}\{x\{t\}\} &= \langle x(t),x(t)\rangle \\ &= \lim_{s\to 1} \partial_s^2 G(s,t) + \langle x(t)\rangle - \langle x(t)\rangle^2 \\ &= \left(\frac{1}{M}\sum_{j=0}^{M} j^2 - \frac{(M+1)^2}{4}\right) + \lambda t = \delta + \lambda t, \end{aligned} \tag{24}$$

$$\delta = \left(\frac{1}{M}\sum_{j=0}^{M} j^2 - \frac{((M+1)^2}{4}\right) = \frac{M^2-1}{12}.$$

Since $\frac{1}{M}\sum_{j=0}^{M} j^2 = \frac{(M+1)(2M+1)}{6}$,

the relation $\lim_{s\to 1} \partial_s^2 G(s,t) = \langle x(t)[x(t)-1]\rangle$ was used. Thus function $h(t)$ that is a measure of the extent of nonreactive dynamics (fluctuations) has the following properties:

$$\langle f,h\rangle = \langle e,h\rangle = 0, \quad \langle h_i,h_i\rangle = \epsilon(\delta + \lambda t) = \delta' + \lambda' t, \tag{25}$$

where ϵ is the corresponding spectroscopic conversion factor (e.g., molecular extinction coefficient). Using the same assumptions about the function $e(t)$, the corresponding sample size autocorrelation function for Eq. (16) can be given as

$$G(\tau,n) = \frac{\langle f_i, f_{i+\tau}\rangle}{\left[\left(\langle f_i,f_i\rangle + \langle h_i,h_i\rangle + \frac{1}{n}\right)\times\left(\langle f_{i+\tau},f_{i+\tau}\rangle + \langle h_{i+\tau},h_{i+\tau}\rangle + \frac{1}{n}\right)\right]^{0.5}}. \tag{26}$$

Putting the value of $\langle h,h\rangle$ from Eq. (25) and taking the limit as in Eq. (7), we obtain the sample size autocorrelation function in the presence nonreactive modes of dynamics as follows:

$$\begin{aligned} G(\tau_0,n) &= \frac{\langle f,f\rangle_{\tau\to 0}}{\langle f,f\rangle_{\tau\to 0} + \delta' + (\lambda'\Delta t)n + \frac{1}{n}} \\ &= \frac{\langle f,f\rangle_{\tau\to 0}}{\langle f,f\rangle_{\tau\to 0} + \delta' + \zeta n + \frac{1}{n}}, \end{aligned} \tag{27}$$

where the relation $t=(\Delta t)n$ was used. Here Δt is the time difference between two consecutive data points, which is constant and $\zeta=\lambda'\Delta t$. But one should also note that $G(\tau_0,n)^{\infty}=0$ and not equal to unity as in Eq. (8), which clearly shows the turn over behavior of the function given by Eq. (27). The same theory can be extended to dynamics under any kind of arbitrary potential too. When f is a constant function (here it can be the blank trial in an experiment), then by definition $\langle f,f\rangle_{\text{constant}} = \langle ff\rangle - \langle f\rangle\langle f\rangle = 0$ and therefore $G(\tau_0,n)=0$ holds for all values of n for a constant function.

EXPERIMENTAL METHODOLOGY

In order to check the validity of Eqs. (7) and (27), conformational transition involved in DNA renaturation was chosen (another possible reaction system could be the unfolding of proteins). Earlier studies[11] clearly showed that it comprised of two phases, namely (1) correct contact forming (following a second order kinetic) and (2) zipping (behaves like a fist order or cooperative process depend on the type of DNA). Even though the first phase behaves ideally (because, here the initial condition is same for all molecules), it leads to randomness of second zipping phase (since the correct contact can occur at any place the entire stretch of DNA, the initial condition is different for different molecules and thus the trajectories of second phase are heterogeneous). Since our interest was to show the heterogeneous nature of second-zipping phase, which occurred in a rate slower (occurs in seconds time scale) than the first one (occurs in subsecond time scale), it was enough to collect data in seconds time scale. The aim would be fulfilled when we showed that fluctuations due to nonreactive modes were negligible (i.e., $1 \gg \lambda$). For this purpose pBR322 plasmid DNA (circular) was purchased from Pharmacia. The size of the plasmid was 4.632 Kbp.[12] Measuring the ratio of absorbance at 260 nm to 280 nm, which was nearly 2, checked the purity. Treating the circular plasmid with HindIII (purchased from Roche chemicals) did the linearization. 1 nM (=2 nM of [ssDNA]) of linearized DNA was used in all the experiments. Raising the temperature to 95 °C in 1×Tris-EDTA buffer *p*H 8.0 did the melting of DNA. Possibility of aggregation was ruled out by preliminary gel filtration studies (data not shown). The time evolution of absorbance at 260 nm was measured by using the Shimadzu-UV2100 spectrophotometer with suitable blank. The data fitting (by nonlinear least square method) was done using the SigmaPlot-5software. The sample size autocorrelation function of the observed absorbance data was calculated using the following relation with fixed τ_0 as:

$$G(\tau_0,n)=\frac{\sum_{i=0}^{n} g_i g_{i+\tau_0}-\left(\frac{\sum_{i=0}^{n} g_i \sum_{i=0}^{n} g_{i+\tau_0}}{n_0}\right)}{\sigma_{g_i}\sigma_{g_{i+\tau_0}}}, \qquad (28)$$

where

$$\sigma^2_{g_{i+\tau_0}}=\sum_{i=0}^{n} g^2_{i+\tau_0}-\frac{\left(\sum_{i=0}^{n} g_{i+\tau_0}\right)^2}{n_0},$$

$$\sigma^2_{g_i}=\sum_{i=0}^{n} g^2_{i}-\frac{\left(\sum_{i=0}^{n} g_{i}\right)^2}{n_0}.$$

Here $\tau_0<n<(N-\tau_0)$.

The method is as follows: For example, let us assume following data points:

$t(s)$	0	1	2	3	4	5	6	7	8	9	10	11	12	13
g	0.4	0.38	0.35	0.37	0.34	0.32	0.33	0.31	0.29	0.30	0.27	0.25	0.23	0.22

Here $N=13$, $\Delta t=1$ s, $g=A^{260}$ (absorbance at 260 nm) and t is the time in seconds. To calculate $G(2,5)$ the required data points can be rewritten as (here delay τ is 2 and $n=5$):

g_i	0.40	0.38	0.35	0.37	0.34
g_{i+2}	0.35	0.37	0.34	0.32	0.33
i	0	1	2	3	4

Now using Eq. (28) it is easy to verify that $G(2,5)=0.501$. Subsequently in the same way, for $n=6,7,8,...(N-2)=11$, $G(2,n)$ has to be calculated and thus the whole autocorrelation function can be constructed and plotted as $G(2,n)$ versus n. The analysis indicated that the sample size autocorrelation [constructed by the aforementioned method with $\tau_0=4$ (=0.8 s), $\Delta t=0.2$ s] of the absorbance data fitted to Eq. (27) with a chi-square value of 0.78 did not fit into Eq. (7). The obtained parameters corresponding to Eq. (27) were $\zeta=1.1627\times10^{-5}$, $\langle f,f\rangle_{\tau=0.8}=0.220$ and $\delta'=3.185\times10^{-3}$. From this ζ value, the approximate λ' was calculated (using the relation $\lambda'=\zeta/\Delta t$) to be 5.81×10^{-5}. So $1\gg\lambda$ indirectly indicated that conformational domains of ssDNA were weakly coupled and thus the path of the subsequent zipping phase was highly heterogeneous which agreed well with earlier studies.[11] Fitted data have been shown in Fig. 2 [$G(4,n)$ versus n] and here the ideal one (Case I) was calculated using Eq. (7) with $\langle f,f\rangle_{\tau_0=0.8}=0.22$. These results suggested that once a correct contact formed, intermolecular interactions between renaturing duplex strands were negligible. In order to confirm this theory one has to check the validity of $\langle f,f\rangle_{\tau=0.8}$. But the complete probabilistic description of DNA renaturation kinetics has not yet been done. The main drawback of this method is: we can get only the overall heterogeneity and not at a single molecule level. Since the input for this analysis is the usual kinetic data, the obtained information is much more economic with respect to that and the obtained parameters can be used to compare the efficiency of two kinetic processes leading to the same product.

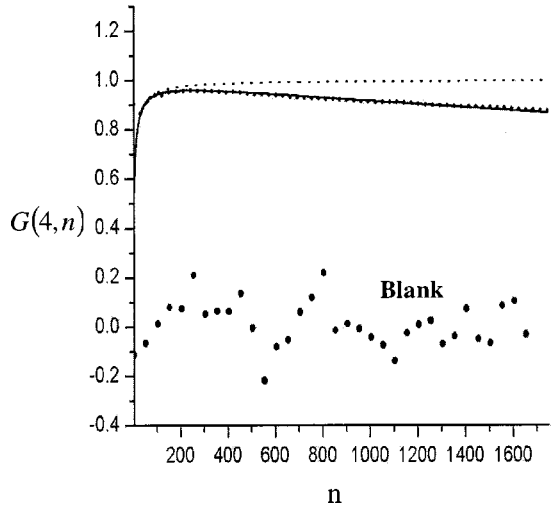

FIG. 2. $G(4,n)$ of renaturation kinetic data of linearized pBR322 DNA. Here small-filled circular dots represent the calculated $G(4,n)$ values from the renaturation data using Eq. (28 and the solid line is the predicted one by Eq. (27). The dotted line is the ideal one (Case I) predicted by Eq. (7) using $\langle f,f\rangle_{\tau_0=0.8}=0.22$ and the blank is without DNA.

CONCLUSIONS

A simple method of analysis using sample size autocorrelation functions to identify and quantify the path heterogeneity in reactions involving macromolecules like biopolymers was developed and applied to DNA renaturation kinetics problem. The results clearly indicate that the path of the so-called (second) zipping phase was heterogeneous (i.e., the trajectory of each renaturing molecule is significantly different from each other), which agreed well with earlier works.

ACKNOWLEDGMENTS

The author thanks Professor S. Mazumdar and Professor S. Mitra for constant encouragement and help.

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