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A stochastic model on DNA renaturation kinetics

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Abstract

A simple stochastic model on DNA renaturation kinetics in the presence and absence of cooperativity have been developed [the corresponding deterministic models have been explicitly treated in our previous work. Biochem Biophys Res Commun 293 (2002) 870–873]. Theoretical mean and variance of number of bases in single-stranded DNA (ssDNA), (which is of course a random variable) have been calculated and compared with the experimental values. The results showed that only the cooperative model correctly predicted the time t_m at which variance becomes maximum whereas, the non-cooperative model overestimated it and thus proved the validity of the cooperative model. Some of the applications of this cooperative theory in resolving the problems of the central dogma of life, PCR etc. have also been discussed.

Keywords: DNA renaturation; Stochastic models; Central dogma; Cooperativity

1. Introduction

The denaturation and renaturation of complementary strands of DNA is an important phenomenon in molecular biology, especially in $C_o t$ analysis [1–3], PCR and denaturing high-performance liquid chromatography (HPLC) [4]. Several mathematical models have been developed to describe the kinetics of DNA renaturation in solution and also have been experimentally tested [5–8]. Conventionally, the renaturation of ssDNA was described by a second order kinetics process as follows:

$$2[\text{ssDNA}] \xrightarrow{k_1} [\text{dsDNA}]$$

Where k_1 ($\text{mol}^{-1}\ \text{s}^{-1}$) is the bimolecular rate constant, [ssDNA] is the concentration (mol/l) of single-stranded DNA and [dsDNA] is the concentration (mol/l) of double stranded DNA and the corresponding differential (rate) equation becomes:

$$-\frac{\text{d[ssDNA]}}{\text{d}t} = k_1[\text{ssDNA}]^2 \tag{1}$$

The formal solution of Eq. (1) for the initial condition at $t=0$, $[\text{ssDNA}]=a_0$ can be given as:

$$[\text{ssDNA}] = \frac{1}{1+k_1 a_0 t}, \quad [\text{dsDNA}] = \frac{k_1 a_0 t^2}{1+k_1 a_0 t} \tag{2}$$

$C_o t_{1/2}$ is an important parameter obtained from

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renaturation studies, where $C_o t_{1/2} = 1/k_1$, $C_0 = a_0$, (i.e. initial concentration of ssDNA in mol/l and $t_{1/2} = 1/k_1 C_0$ is the time t to obtain [ssDNA] = $C_0/2$], which is used to find the 'complexity' (a term used to denote the unique number of base-pairs contained in a genome and its repetitive nature) of a given genome. However, recent studies [9] have shown that the renaturation was not just a simple second order process but contained a correct contact-forming phase (which is bimolecular and fast), followed by an intramolecular zipping phase (which is unimolecular and slow) and the correct order parameter to describe the renaturation process was neither [dsDNA] nor [ssDNA] but the 'number of mol-bases' in ssDNA or dsDNA. It was also shown that the theoretical $C_o t$ values obtained from cooperative models agreed fairly well with the experimental $C_o t$ values whereas non-cooperative models underestimated the $C_o t$. Moreover, the sample-size autocorrelation analysis of kinetic-data of renaturation, (i.e. time evolution of absorbance at 260 nm) showed that the zipping-molecules followed a heterogeneous path, (i.e. a stochastic process [10]) and also showed that the so-called 'connectedness' of conformational domains [here connectedness between two conformational domains A and B denotes the probability (P) of finding a molecule simultaneously in both of them, i.e. P (A$\bigcap$B)] of ssDNA was much less than unity. Therefore, we can conclude that the renaturation process is homogenous and deterministic in the macroscopic sense, (i.e. the correct contact forming phase) but heterogeneous in the microscopic sense (zipping phase), i.e. a mesoscopic phenomenon where the system follows stochastic laws rather than deterministic laws. Aforementioned arguments show that in the process of renaturation, the number of bases in dsDNA or ssDNA is a stochastic quantity, (i.e. the zipping phase prone to higher fluctuation and, therefore, it is a slow process). Due to this reason, stochastic analysis of DNA renaturation is necessary to understand the process at a microscopic level and also to optimize the protocols, which are based on renaturation, (e.g. in PCR, the annealing time should be optimized in such a way that zipping of a primer with its complementary region of template DNA is complete and the fluctuation is minimum, which will otherwise lead to a non-specific amplification and less yield). In this article, a stochastic model of DNA renaturation has been developed and with its help, the presence of cooperativity in the zipping of complementary strands confirmed and some of its potential applications have also been discussed.

2. Stochastic theory of DNA renaturation kinetics

As we argued earlier, the correct contact-forming phase follows a second order kinetics and the zipping phase follows a first order kinetics. Thus, the set of (deterministic) coupled rate equations describing renaturation process can be written as follows [9]:

For non-cooperative zipping:

$$\begin{aligned} \mathrm{d}_t x &= -k_1 x^2 \\ \mathrm{d}_t N &= -k_2 \times (a_0 - x) \times N \end{aligned} \tag{3}$$

For cooperative zipping:

$$\begin{aligned} \mathrm{d}_t x &= -k_1 x^2 \\ \mathrm{d}_t N &= -k_2 \beta \times (N_0 - N) \times (a_0 - x) \times N \end{aligned} \tag{4}$$

Where x = [ssDNA] (mol/l), a_0 = [ssDNA] $t = 0$, (i.e. concentration of ssDNA at time $t = 0$ in mol/l), N is the number of bases in ssDNA, k_1 is the bimolecular rate constant ($\mathrm{mol}^{-1}\ \mathrm{s}^{-1}$) k_2 in the unimolecular rate constant (mol-base^{-1}) and $\beta = 1/N_0$ (mol-base^{-1}) is the cooperativity index. Here $(a_0 - x)$ in the expression of $\mathrm{d}_t N$ denotes the mol-number of correct contacts (we are multiplying by this to account for all zipping initiated molecules at time t) symbolically and $(N_0 - N)$ (bases in dsDNA) denotes the cooperative effect of already zipped bases. Assuming $\{N,x\} \in Z$, (i.e. integer), the stochastic analogs of Eqs. (3) and (4) can be derived as follows: since the correct contact-forming phase is much faster than zipping phase, i.e. $\Delta_x t \ll \Delta_N t$ and $\lim_{\Delta_x t, \Delta_N t \to 0} (\Delta_x t / \Delta_N t) = 0$ (this can happen only and only when $\Delta_x t$

approaches zero faster than $\Delta_N t$) the following limit should hold:

$$\lim_{\Delta_N t} x = \langle x \rangle_{\det} = \frac{1}{1 + k_1 a_0 t} \tag{5}$$

Here the subscript 'det' denotes the deterministic limit and this method of eliminating fast variable is called as 'adiabatic elimination' in stochastic theory [11]. However, this is slightly different from a standard procedure in a way that here renaturation is a non-stationary process and due to this fact we have taken the deterministic limit to eliminate the fast variable x (but still it is a function of time!). Now the birth–death master equation for the probability distributions $P(x, t)$ and $P(N, t)$ can be written as follows.

$$\begin{aligned}\partial_t P(x,t) &= \lim_{\Delta_x t \to 0} \frac{P(x,t+\Delta_x t) - P(x,t)}{\Delta_x t} \\ &= k_1 \big[(x+2) \\ &\quad \times (x+1) P(x+2,t) - x(x-1) P(x,t) \big]\end{aligned} \tag{6}$$

For non-cooperative zipping:

$$\begin{aligned}\partial_t P(N,t|\bar{z} \geq 1) &= \\ &\lim_{\Delta_N t \to 0} \frac{P(N,t+\Delta_N t|\bar{z} \geq 1) - P(N,t|\bar{z} \geq 1)}{\Delta_N t} \\ &= k_2 \times \bar{z} \times \big[(N+1) P(N+1,t|\bar{z} \geq 1) \\ &\quad - N \times P(N,t|\bar{z} \geq 1) \big]\end{aligned} \tag{7}$$

And for cooperative zipping:

$$\begin{aligned}&\partial_t P(N,t|\bar{z} \geq 1) \\ &= \lim_{\Delta_N t \to 0} \frac{P(N,t+\Delta_N t|\bar{z} \geq 1) - P(N,t|\bar{z} \geq 1)}{\Delta_N t} = k_2 \beta \\ &\quad \times \bar{z} \times \big[(N+1)(N_0 - N - 1) P(N+1,t|\bar{z} \geq 1) \\ &\quad - N(N_0 - N) \times P(N,t|\bar{z} \geq 1) \big]\end{aligned} \tag{8}$$

Where, $P(x, t)$ denotes the probability of finding x number of ssDNA molecules at time t and $\bar{z} = \langle a_0 - x \rangle_{\det}$ denotes the mean number (here it is equal to deterministic limit) of correct contacts [it is equal to the concentration of dsDNA in conventional models, i.e. Eq. (2)] at time t, $P(N, t|\bar{z} = 1, t)$ denotes the conditional probability of occurrence of N number of bases in ssDNA form at time t, provided that $\bar{z} \geq 1$ at time t. The aforementioned condition was insisted due to the fact that in order to initiate the zipping process at least one correct contact was necessary. The differential-difference Eqs. (7) and (8) can be solved as follows (we are neglecting the master equation for x, since it is a fast variable):

2.1. Case I: no cooperativity in zipping:

Using $P(N,t|\bar{z} \geq 1,t) = P(N,t;\bar{z} \geq 1,t)/P(\bar{z} \geq 1,t)$, Eq. (7) can be rewritten as:

$$\begin{aligned}\partial_t P_N &= k_2' \bar{z} \times \big[(N+1) P_{N+1} - N \times P_N \big] + P_N \\ &\quad \times \partial_t \big[\ln P(\bar{z} \geq 1,t) \big]\end{aligned} \tag{9}$$

Where $P_N = P(N,t;\bar{z} \geq 1,t), P_{N+1} = P(N+1,t;\bar{z} \geq 1,t)$.

Defining, $G(s,t) = \sum_{N=0}^{N_0} S^N P_N$, Eq. (9) becomes:

$$\begin{aligned}\partial_t G(s,t) &= k_2 \bar{z} \times (1-s) \partial_s G(s,t) \\ &\quad + \partial_t \big[\ln P(\bar{z} \geq 1,t) \big] G(s,t)\end{aligned} \tag{10}$$

Here N_0 denotes the total number of bases in ssDNA form at time $t = 0$, N (number of bases in ssDNA) is the random variable of our interest and $\bar{z}$ is the number of correct contacts that has already occurred. The solution of Eq. (10) is the following arbitrary function F:

$$G(s,t) = F\left[(1-s) \times P(\bar{z} \geq 1,t) \times \exp\left\{ -k_2 \int \bar{z} \mathrm{d}t \right\} \right] \tag{11}$$

Using the following initial condition [here one should note that P ($\bar{z} = 1$, 0)1] which is due to

adiabatic elimination of fast variable x):

$$G(s,0)=F\left[(1-s)\right]=s^{N_0}$$

$$G(s,t)=\left[1-(1-s)\times\exp\left\{-k_2\int\bar{z}\mathrm{d}t\right\}\right]^{N_0} \tag{12}$$

Now from Eq. (12) the mean and variance of N can be calculated as follows:

$$\begin{aligned}\langle N\rangle_t^{\mathrm{I}}=\overline{N}^{\mathrm{I}}&=\lim_{s\to 1}\partial_s G(s,t)\\&=N_0\times e^{-k_2a_0t}\times(1+k_1a_0t)^{k_2/k_1}\end{aligned} \tag{13}$$

And one should note that $\lim_{k_2/k_1\to 0}[t_{1/2}^{\mathrm{I}}]=\ln 2/k_2a_0$ where $t_{1/2}^{\mathrm{I}}$ denotes the time at which $\langle N\rangle_t^{\mathrm{I}}=N_0/2$.

$$\begin{aligned}\mathrm{Var}^{\mathrm{I}}\{N\}_t&=\lim_{s\to 1}\partial_s^2G(s,t)+\overline{N}^{\mathrm{I}}-(\overline{N}^{\mathrm{I}})^2\\&=N_0\times(1+k_1a_0t)^{k_2/k_1}\times\mathrm{e}^{-k_2a_0t}\\&\quad\times(1-(1+k_1a_0t)^{k_2/k_1}\times\mathrm{e}^{-k_2a_0t})\end{aligned} \tag{14}$$

Here $\langle N\rangle_t^{\mathrm{I}}$ denotes the mean number of bases in ssDNA form at time t and the superscript 'I' denotes the case I. One also should note that the variance has a maximum at:

$$\lim_{k_2/k_1\to 0}t_{\mathrm{m}}^{\mathrm{I}}=\frac{\ln 2}{k_2a_0} \tag{15}$$

Therefore, in the absence of cooperativity the equality $t_{1/2}^{\mathrm{I}}=t_{\mathrm{m}}^{\mathrm{I}}$ holds.

2.2. Case II: in the presence of cooperativity

Performing same calculations as in case I, we obtain:

$$\begin{aligned}\partial_tP_N=k_2'\bar{z}\times(N_0-N)\times\left[(N+1)P_{N+1}-N\times P_N\right]\\+P_N\times\partial_t\left[\ln P(\bar{z}\geq 1,t)\right]\end{aligned} \tag{16}$$

Here $P_N=P(N,t;\bar{z}\geq 1,t)$, $P_{N+1}=P(N+1,t;z\geq 1,t)$, $k_2'=k_2\beta$ (mol-base^{-2} s^{-1}) and $\beta=1/\mathrm{N}_0$ (mol-base^{-1}) is the cooperativity index.

$$\begin{aligned}\partial_tG(s,t)=k_2'\bar{z}\times\{&(N_0-1)(1-s)\times\partial_sG(s,t)\\&-s(1-s)\partial_s^2G(s,t)\}\\&+\partial_t\left[\ln P(\bar{z}\geq 1,t)\right]G(s,t)\end{aligned} \tag{17}$$

Putting $G(s,t)=S(s)\times T(t)$ in Eq. (17) and assuming $P(\bar{z}\geq 1,t_1)\approx P(\bar{z}\geq 1,0)=1$, we obtain:

$$T_n(t)=\mathrm{e}^{-k_2'a_0nt}\times(1+k_1a_0t)^{k_2'n/k_1} \tag{18}$$

$$\begin{aligned}S_n(s)=s^{N_0}\times{}_2F_1(&a_n+N_0,b_n+N_0;a_n+b_n\\&+N_0;1-s)\end{aligned} \tag{19}$$

$S_n(s)$ is the solution of $s(1-s)d_s^2S+(N_0-1)\times(s-1)d_sS-nS=0$, and

$$b_n=\frac{-N_0+\sqrt{N_0^2-4n}}{2},\quad a_n=\frac{2n}{-N_0+\sqrt{N_0^2+4n}},$$

$$c=-(N_0-1)$$

Here ${}_2F_1$ is the hyper-geometric function. For the initial condition $G(s,0)=F\left[\sum_{n=0}^{N_0}S_n(s)\right]=s^{N_0}$ the solution to Eq. (17) becomes as (by Lagrange expansion, [12]:

$$\begin{aligned}G(s,t)=\Bigg\{1+\sum_{k=1}^{\infty}&\frac{(S_T(s)-N_0)^k}{k!}\\&\times\left[\frac{\mathrm{d}^{k-1}}{\mathrm{d}s_{k-1}}\left\{\frac{s-1}{S_T(s)-N_0}\right\}^k\right]_{s=1}\Bigg\}^{N_0}\end{aligned} \tag{20}$$

Where $S_T(s)=\sum_{n=0}^{N_0}S_n(s)\times T_n(t)$ and now $P(N,t)$ can be given as:

$$\begin{aligned}P(N,t)=\Bigg\{1+\sum_{k=1}^{\infty}&\frac{(S_T^N(1)-N)^k}{k!}\\&\times\left[\frac{\mathrm{d}^{k-1}}{\mathrm{d}s_{k-1}}\left\{\frac{s-1}{S_T^N(s)-N}\right\}^k\right]_{s=1}\Bigg\}^{N_0}\end{aligned} \tag{21}$$

Where $S_T^N(1)=\sum_{n=0}^{N}T_n(t)$. The mean and variance of the random variable N in the presence of cooperativity can be given as:

$$\langle N\rangle_t^{\mathrm{II}} = \lim_{s\to 1}\partial_s G(s,t) = N_0\sum_{k=1}^{\infty}\frac{(S_T(1)-N_0)^{k-1}}{(k-1)!} \times\left[\left\{\frac{\mathrm{d}}{\mathrm{d}s}\left[S_T(s)\right]\right\}\frac{d^{k-1}}{ds_{k-1}}\left\{\frac{s-1}{S_T(s)-N_0}\right\}^k\right]_{s=1} \quad (22)$$

To a crude approximation we can write $\langle N\rangle_t^{\mathrm{II}} = \sum_{n=0}^{N_0} T_n(t)$ and at large values of N_0 (noting that $n = N_0$ in deterministic case [9], Eq. (22) can be simplified to:

$$\langle N\rangle_t^{\mathrm{II}} = \overline{N}^{\mathrm{II}} \approx \sum_{k=0}^{\infty}(T_{N_0}(t))^k = \frac{1}{1-T_{N_0}(t)} \quad (23)$$

Also one should note that $\lim_{k_2'/k_1\to 0}[t_{1/2}^{\mathrm{II}}] = \ln 2/k_2' a_0$, where $t_{1/2}^{\mathrm{II}}$ is the time at which $\langle N\rangle_t^{\mathrm{II}} = N_0/2$.

$$\mathrm{Var}^{\mathrm{II}}\{N\}_t = \lim_{s\to 1}\partial_s^2 G(s,t) + \overline{N}^{\mathrm{II}} - (\overline{N}^{\mathrm{II}})^2 = \sum_{n=0}^{N_0} T_n(t) \times\left\{N_0 - \sum_{n=0}^{N_0} T_n(t)\right\} \quad (24)$$

$$\lim_{k_2'/k_1\to 0}\{\mathrm{Var}^{\mathrm{II}}\{N\}\} \approx \frac{N_0 - N_0 T_{N_0}(t) - 1}{(1-T_{N_0}(t))^2} \quad (25)$$

Therefore, the variance of N in the presence of cooperativity has a maximum at [using approximation given by Eq. (23)]:

$$\lim_{k_2'/k_1\to 0}\{t_{\mathrm{m}}^{\mathrm{II}}\} = \frac{1}{k_2' a_0 N_0}\ln\frac{N_0}{N_0-2} \quad (26)$$

Here superscript 'II' denotes case II, $t_{\mathrm{m}}^{\mathrm{II}} = \left(t_{1/2}^{\mathrm{II}} \times \ln\left[N_0/(N_0-2)\right]\right)$ and $t_{1/2}^{\mathrm{II}} \gg t_{\mathrm{m}}^{\mathrm{II}}$.

3. Experimental materials and methods

In order to confirm the validity Eqs. (15) and (26) renaturation kinetic experiments were conducted on a linearized plasmid DNA as described in earlier works [9]. The pBR322 plasmid DNA (circular) was purchased from Pharmacia. The size of the plasmid was 4.632 Kbp [13]. 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. One nanomole (=2 nM of [ssDNA]) of linearized DNA was used in all the experiments. Raising the temperature to 95 °C in 1× Tris–EDTA buffer pH 8.0 melted the DNA. Possibility of aggregation was ruled out by preliminary gel filtration studies (data not shown). The renaturation was monitored at 260 nm (decrease in absorbance, i.e. hypochromic effect) by Shimadzu-UV2100 spectrophotometer with a suitable blank. The time evolution data was collected for 1 h at 0.2-s intervals (Δt), (i.e. 18 000 data points). Since the experimental data (absorbance at 260 nm) was a discrete one (here $A_{260} \propto N$ where N is the mol-bases in ssDNA and thus $\mathrm{Var}\{N\} \propto \mathrm{Var}\{A_{260}\}$), we could use the following equation to calculate the variance.

$$\mathrm{Var}^{\mathrm{obs}}\{N\}_t = \frac{\sum_{i=0}^{t/\Delta t} N_{i\Delta t}^2 - \frac{\left(\sum_{i=0}^{t/\Delta t} N_{i\Delta t}\right)^2}{t/\Delta t}}{(t/\Delta t)-1} \quad (t/\Delta t) > 1 \quad (27)$$

Here N_i denotes the ith data point where $i = t_i/\Delta t$, $1 < i < 18\,000$ and $i \in Z$, (i.e. an integer). The method is as follows: for example let us assume the following data points of time evolution.

t_i (s)	0	1	2	3	4
N_I	0.60	0.55	0.56	0.51	0.49
t_i (s)	5	6	7	8	9
N_I	0.48	0.49	0.47	0.45	0.46

Here $\Delta t = 1$ s, total number of data points is 10, $N_1 = 0.6$, $N_2 = 0.55$ and so on. From Eq. (27) it is easy to verify that $\mathrm{Var}^{\mathrm{obs}}\{N\}_{t=4s} = 1.87\times 10^{-3}$ and for $t = 5$ s, its 2.14×10^{-3} and so on. In the same way, the whole variance function has to be constructed from which the time t_{m} can be calculated (just by observing the maximum).

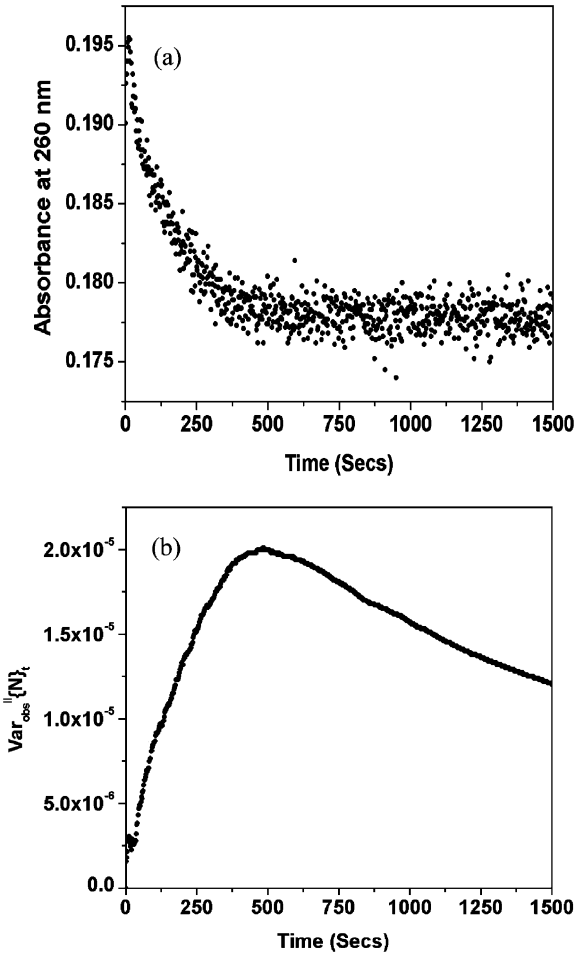

Fig. 1. (a) This figure shows the spectroscopic evolution of renaturation of linear pBR322 plasmid, (i.e. absorbance measured at 260 nm with an initial [ssDNA] concentration of 2 nM and here the $\Delta t = 0.2$ s) with time. (b) This figure shows the constructed variance [as described in Section 3 using Eq. (27)] that shows a maximum (t_m) at 450 s.

4. Results and discussions

The observed spectroscopic evolution of renaturation, (i.e. absorbance at 260 nm) and the corresponding constructed variance have been shown in Fig. 1a,b. The theoretical t_m values in absence and presence of cooperativity were calculated using Eqs. (15) and (26) (here $k_2 = 2.2\times10^4$ mol^{-1} s^{-1}, $k_2' = 0.128$ mol^{-2} s^{-1} (these values were taken from earlier kinetic studies [9]), $a_0 = 2$ nM and $N_0 = 4.632\times10^3$ bases). The obtained theoretical values were: $t_m^{I} = 1.58\times10^4$ s and $t_m^{II} = 364$ s but the experimental t_m was 450 s (as could be seen from Fig. 1). Therefore, the t_m value obtained from cooperative model was nearly close to the experimental value [here the difference (approx. 100 s) may be due to the approximation done in Eq. (23)] and thus confirmed its validity. The time at which the occurrence of maximum in the variance of random variable N is an important parameter due to the fact that if $t_{1/2}$ (this is the parameter which decides the value of C_0t) coincides with $t_m^{I/II}$ then it is prone to higher fluctuations, (i.e. error), which will introduce to a great uncertainty in C_0t. Comparison of $t_{1/2}$ and t_m showed that renaturation with cooperativity is superior than renaturation in its absence. Generally, the PCR (polymerase chain reaction) will have a three-step temperature cycle viz. melting (approx. 92 °C) of complementary strands of template DNA, annealing of primers with their recognition sites (at 55 °C) and the extension (at approx. 72 °C and it is the real polymerization phase). Here, the annealing phase is the one which decides the 'specific amplification and yield' of fragment of our interest. Prolonged annealing time will generally result in a non-specific amplification, (i.e. amplification of part of the template DNA which is not of interest to us). Therefore, this annealing time should be decided by the lifetime (τ_a) of primer-template zipping. In this context, the cooperative model predicts the annealing time more accurately (and correctly) than non-cooperative models (according to our model the annealing time (t) should fall in the range $t_m \ll t < \tau_a$). This is not only important in the application point of view (like in PCR) but also important in resolving the problems of 'central dogma', (i.e. replication, transcription and translation of the genetic code where the initial step is invariably the recognition of a consensus sequence of DNA by its complementary DNA (in DNA replication) and RNA (in transcription and translation) of life too. Extensive studies have shown that the so-called 'initiation' step involved two clear phases namely: a random search (its generally biased and fast) of complementary molecules [ribosome, (i.e. rRNA) etc.] for their consensus sequence of DNA which was prone to

higher fluctuations; and followed by a slow phase with higher 'processivity' (means lower fluctuation). Our analysis showed that only case II satisfied this condition, (i.e. $t_{1/2} \gg t_m$) and thus we can conclude that renaturation of complementary strands of DNA is a cooperative phenomenon.

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