Thermal Fluctuations of DNA Transcription Enzymes

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An analytical application of the 1-D Brownian dynamics in a fluid is used to study the thermal dynamics of bio-enzymes. The origin of thermal fluctuations that give rise to mutations during the conduct of DNA test using imaging techniques was identified and a solution to carry out the techniques without thermal fluctuations of the bio-enzymes has been suggested. The Langevin equation, the consistency of the equipartition of the kinetic energy theorem and the fluctuations of the positions 1-D Brownian particle in the fluid were used. Also, the results on previous work on thermal dynamics of bio-enzymes were reproduced analytically and used to plot the mean squared displacement (MSD) against time. Our results give a straight-line graph for isotropic random-walk, a curve facing downwards for confined random-walk, a three point segment graph for unrestricted and isotropic random-walk and finally a zigzag line graph for partially confined random-walk. Comparative studies of the above results with that of Aleksandra Radenovic are in good agreement. However, the result only differs in a partially confined random-walk, where Aleksandra Radenovic had a hopping nature of graph for the 2-D Brownian dynamics.

1. Introduction

In bio-genetics, the conduct of DNA test using imaging techniques confronts the geneticists and bio-engineers with fundamental limits due to thermal fluctuations of DNA transcription enzymes resulting from random collisions by the surrounding molecules of the acid fluid. This problem of thermal motions gives rise to DNA mutations in the absence of genetic traits, i.e., variations or distortion of the imaging structure of the chromosomes of an offspring from that of the parents. As such the geneticists cannot ascertain with up to 70% accuracy whether the offspring is a true child of the parents or not.

In the past, many imaging techniques have been devised that enabled the conduct of DNA test. Such scientific techniques include the Scanning Tunnelling Microscopes (STM**)** [1], the Co focal Laser Scanning Microscopes and the near field Scanning Optical Microscopy [2]. These techniques have made it possible to study the dynamical behaviour of some individual functional bio-systems, such as the mechanics of intracellular cytoskeleton network [3], the membrane proteins movement on the surface of living cell [4] and DNA transcription enzymes [5].

However, the accuracy of these techniques has been hijacked by fundamental limits due to thermal fluctuations [6]. Also attempts to obtain DNA sequence information by measuring the rupture forces upon unzipping the strands of single DNA molecule are hampered by thermal motions of the two single strands formed [7]. This problem of thermal motions gives rise to DNA mutations, i.e., variations or distortion of the chromosomal structure of the offspring from that of the parents.

These techniques have been advanced to single particle tracking (SPT), a novel application to probing DNA conformational dynamics on the level of single molecule carried out by Finzi and Gelles in 1995. The application has opened the possibility of obtaining DNA sequence information without perturbation arising from thermal motions of the transcription enzymes.

However, the current study of models for analyzing the random fluctuation data on SPT is still limited. It includes diffusion with and without drift, in a free space, or in confinement [8], and in complex fluid [9]. Most of the models that are currently in use, embrace theoretical approach that guarantees only local solution, i.e., one that exists in some neighborhood points at which initial value is given. Several classes of this type of models deserve further detailed analysis [11].

In this work, the origin of the problem of thermal motions due to thermal fluctuations

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associated with DNA transcription enzymes that give rise to DNA mutations in the absence of the genetic traits during the conduct of the DNA test using imaging techniques is identified. Also, a solution is suggested to this problem, which has been a major setback to the geneticists and bio-engineers in obtaining DNA sequence information.

To obtain a better physical understanding of the dynamical behavior of individual bio-molecules, a good understanding of thermal fluctuations is needed [10]. The usual starting point is the Langevin equation, whose form is assumed in most text books but without discussion on how the collisions between a Brownian particle and its much lighter surrounding molecules give rise to a dissipative and stochastic force [10]. Also, this article reproduces the simulation work on SPT for the 2-D Brownian dynamics carried out by Aleksandra Radnovic in 2005 for the thermal dynamics of bio-enzymes in active transport and restricted motions of the enzymes in the cells of living organisms.

2. Theoretical Framework

Consider the kinematics of a single collision event between a Brownian particle of mass *M* and surrounding molecules of the fluid of mass *m*.
By conservation of linear momentum and energy, we have Eqns. (1) and (2), respectively

$$Mv_a + mv_b = Mv_a' + mv_b' \tag{1}$$

$$\frac{1}{2}Mv_a^2 + \frac{1}{2}mv_b^2 = \frac{1}{2}Mv_a'^2 + \frac{1}{2}mv_b'^2 \tag{2}$$

The Langevin equation is given as

$$M\frac{dV}{dt} = -\gamma V + F(t) \tag{3}$$

Where, the total force, $M\frac{dV}{dt}$, is acting on the Brownian particle suspended in a fluid. It is the sum of a viscous drag (frictional force − γV) term and a fluctuating random force term *F* (*t*), where γ is the dissipative constant.

The consistency of the equipartition of the kinetic energy theorem is expressed as

$$\frac{1}{2}M\langle v^2\rangle = \frac{1}{2}k_BT \tag{4}$$

The fluctuation-dissipation theorem is given as

$$\langle x^2(t)\rangle = \langle (\Delta x)^2 \rangle = \frac{2K_BT}{\gamma}t \tag{5}$$

Where, $\langle x^2(t)\rangle$ is the mean squared displacement (MSD), K_B is the Boltzmann constant and T is the absolute temperature.

Einstein's theory relating mean squared fluctuation, $\overline{\Delta x}^2$ and the diffusion coefficient D is given as [13]

$$\overline{\Delta x}^2 = 2Dt \tag{6}$$

The diffusion - dissipation relation is

$$\mathrm{D} = \frac{K_BT}{\gamma} \tag{7}$$

Also Stokes law is a given as [14]

$$\gamma = 6\pi a\mu_k \tag{8}$$

Eqn. (8) is used in obtaining the dissipative constant γ of the fluid from its kinematic viscosity μ_k at a particular temperature. Here a is the radius of the Brownian particle suspended in the fluid.

The dissipation-collision relation is given as

$$\gamma = 2mn \tag{9}$$

Where, *m* is the mass of the surrounding molecules of the fluid and *n* is the number of collisions per unit time [6].

3. Analytical Methods and Results

Several computations associated with Eqns. (5), (7) and (8) were carried out analytically. The values obtained were tabulated and plotted using Origin-60 computer package. A comparative analysis of the results with the one simulated from the Single Particle Tracking techniques by Radenovic for thermal dynamics of bio-enzymes in the cells of living organisms (in both active transport and restricted motions of the enzymes) was employed [15].

The following steps were followed

a. Raw data comprising the kinematic viscosities and densities of water at different temperatures of the fluid was collected. (Table 1) [16].

Table 1: Kinematic viscosities and density of water at various temperatures.

Temp. (℃)	Kinematic viscosity, μ_k (Ns/m^2) $\times 10^{-3}$	Density(kg/m^3)
10	1.307	999.7281
15	1.139	999.1286
20	1.003	998.2336
25	0.892	997.0751
30	0.800	995.6782
35	0.723	994.0635
40	0.658	992.2473

b. Values of the dissipative constant**,** γ of the fluid **(**used as first input parameter) with respect to Brownian particle of radii $1\mu m$ from the kinematic viscosity μ_k at different temperatures of the fluid for 1-D Brownian dynamics of radius a = $5\times 10^{-7}m$ were computed using Eqn. (8) (Table 2).

Table 2: Dissipative constant, γ obtained from the kinematic viscosity μ_k.

T (℃)	μ_k (Ns/m^2) $\times 10^{-3}$	$\gamma\ (kg/s) \times 10^{-8}$
10	1.307	1.2323
15	1.139	1.0739
20	1.003	0.9457
25	0.892	0.8410
30	0.800	0.7543
35	0.723	0.6817
40	0.658	0.6204

c. The diffusion coefficient D (used as second input parameter) at different temperatures T of the fluid were computed using Eqn. (7) and the values of $ln\,D$ and $\frac{1}{T}$ were also evaluated. (Table 3).

Table 3: Diffusion coefficient D.

$T(K)$	$\gamma\ (kg/s) \times 10^{-8}$	$D\left(\frac{m^2}{s}\right) \times 10^{-13}$	$\ln D$	$\frac{1}{T} \times 10^{-3}\ (K^{-1})$
283	1.2323	3.1692	-28.7801	3.5336
288	1.0739	3.7009	-28.6250	3.4722
393	0.9457	4.2756	-28.4807	3.4130
298	0.8410	4.8899	-28.3444	3.3557
303	0.7543	5.5434	-28.2210	3.3003
308	0.6817	6.2350	-28.1034	3.2468

d. Values of the mean squared displacement (MSD) for isotropic random walk of the bio-enzymes at a constant thermal bath ($T = 283K\ or$ 10℃) using Eqn. (5) within the time t of motion (0.1 to 0.6 seconds) for a Brownian particle of radii 1.0 μm suspended in the fluid were calculated (Table 4).

Table 4: Values of MSD for isotropic random walk at a constant thermal bath ($T = 283K$ or 10℃).

$t(s)$	$\langle X^2\rangle \times 10^{-13}m^2$
0.1	0.63384
0.2	1.2.6768
0.3	1.9.0152
0.4	2.5.3536
0.5	3.1.6920
0.6	3.8.0304

e. Values of the dissipative constant γ of the fluid at a constant thermal bath (T = 283K or 10℃) for a Brownian particle of different radii ranging from 1.0 to 6.0 μm was used to obtain the corresponding diffusion coefficients D for confined random-walk of the enzymes by using Eqn. (7) (Table 5).

Table 5: Dissipative constant of the fluid (water) at a constant temperature bath (T =283k or 10^{o}C) and the diffusion coefficient, D.

$Diameter\ (\mu m)$	$\gamma(kgs^{-1}) \times 10^{-8}$	$D(m^2/s) \times 10^{-13}$
1.0	1.2323	3.1692
2.0	2.4646	1.5846
3.0	3.6969	1.0564
4.0	4.9292	0.7923
5.0	6.1616	0.6338
6.0	7.3939	0.5282

f. The corresponding values of MSD for the confined random-walk of the enzymes with values of D in Table 5 were computed using Eqn. (5) (Table 6).

Table 6: Values of MSD for the confined random-walk of the enzymes.

$t(s)$	$D(m^2/s) \times 10^{-13}$	$\langle X^2 \rangle \times 10^{-13} m^2$
0.05	3.1692	0.31992
0.15	1.5846	0.47538
0.25	1.0564	0.52820
0.35	0.7923	0.55461
0.45	0.6338	0.57042

g. Values of MSD for partially confined random-walk at different time *t* for Brownian particles of radii; $5\mu m$, $4\mu m$ and $3\mu m$ at a constant thermal bath (T = 10℃ or 283K) were computed using their corresponding diffusion coefficients in Table 6 (Table 7).

h. The raw data obtained i.e., the values of the kinematic viscosities of the fluid (water) at different temperatures was validated by comparing the plot of $ln\ D$ against $\frac{1}{T}$ with the same plot of an already existing relation between D and T given by $D = D_0 e^{-\frac{Q}{RT}}$. Where, D_0 is a temperature independent pre-exponential, Q is the activation energy of diffusion (J/mol), R is the gas constant (8.31 J/mol K^{-1}) and T = Absolute temperature (K) [17]. The table and graph are displayed in Table 4 and Fig. 1, respectively.

i. The plot of MSD versus time for thermal dynamics of bio-enzymes in the cells of living organisms for isotropic random-walk, confined random-walk, isotropic and unrestricted random-walk, and partially-confined random-walk were determined. The results are displayed in Figs. 2a, 3a, 4a and 5a, respectively.

j. The results obtained were compared with the simulated results on SPT in a computer device for 2-D Brownian dynamics to the thermal motions of the bio-enzymes carried out in [15]. The results are shown in Figs. 2b, 3b, 4b and 5b.

k. A proposed solution to thermal fluctuations of the DNA transcription enzymes that give rise to mutations in the absence of the genetic traits on using imaging techniques was then proposed.

Table 7: Values of partially confined random- walk for MSD at time, t for 1-D Brownian particles of radii; $5\mu m, 4\mu m\ and 3\mu m$ respectively.

T(s)	$\langle X^2\rangle \times 10^{-3} m^2$	$\langle X^2\rangle \times 10^{-13} m^2$	$\langle X^2\rangle \times 10^{-13} m^2$
0.05	0.07923	0.06338	0.05252
0.10	0.15846	0.12676	0.10504
0.15	0.23769	0.19014	0.15756
0.20	0.31692	0.25352	0.21008
0.25	0.39615	0.31690	0.26250
0.30	0.47538	0.38028	0.31512
0.35	0.55461	0.44366	0.36764
0.40	0.63384	0.50704	0.42016
0.45	0.71307	0.57042	0.47268
0.50	0.79230	0.63380	0.5252

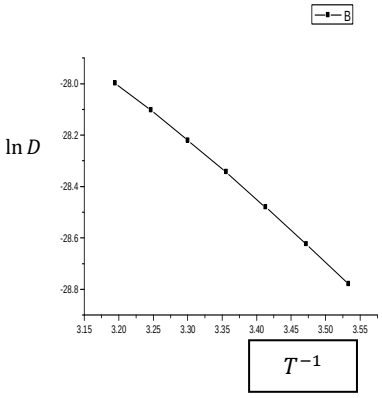

Fig.1a: The graph of *lnD* against $\frac{1}{T}$.

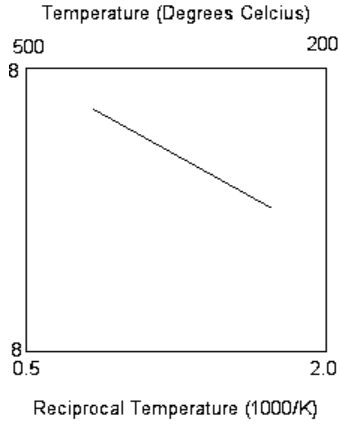

Fig.1b: The simulated plot of lnD against $\frac{1}{T}$.

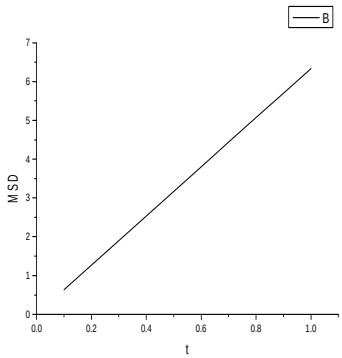

Fig.2a: The plot of MSD vs. time.

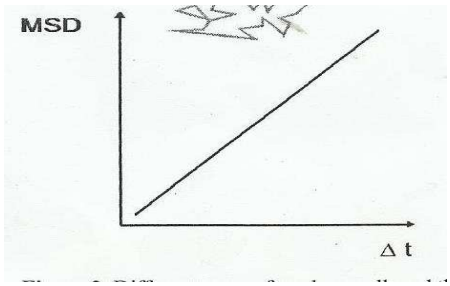

Fig.2b: The isotropic random walk and the corresponding MSD plot against time [15].

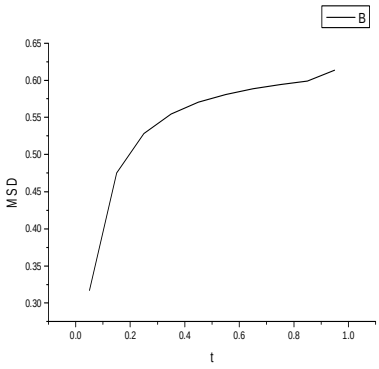

Fig.3a: The plot of MSD vs. time t for confined random-walk.

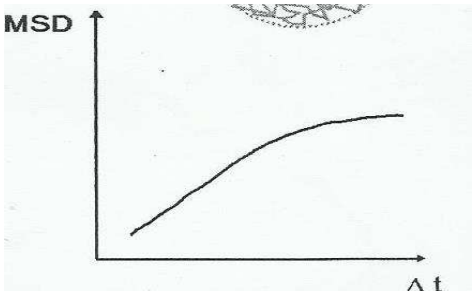

Fig.3b: The simulated result for confined random-walk.

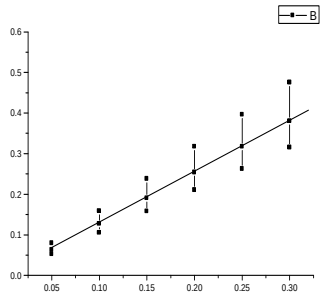

Fig.4a: The plot for unrestricted and isotropic random-walk.

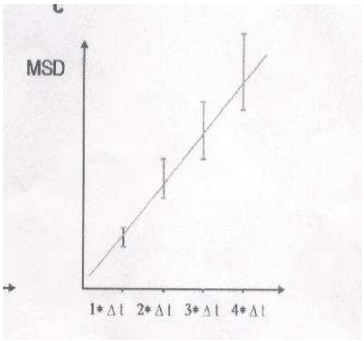

Fig.4b: The corresponding simulated result.

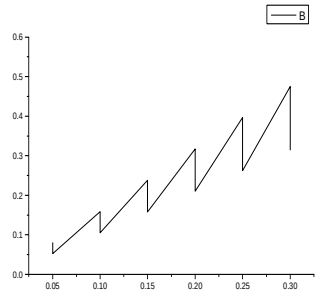

Fig.5a: The plot for partially confined random-walk.

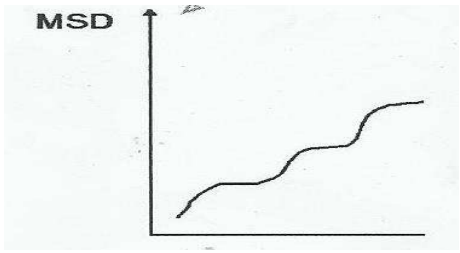

Fig.5b: The simulated result for partially confined random-walk.

4. Discussion of Results

The result of Fig. 1a is not a part of the research work but only implored to validate our raw data in Table 1 and the consistency of first input parameter γ from the kinematic viscosity μ_k using Stokes law. The result is agreement with the simulated result in Fig. 1b. Fig. 2a gives a straight line for the plot of MSD against time. It is an isotropic random walk. The time dependence of MSD gives diffusion coefficient D, which is constant in time, indicating a uniform diffusion for the 1-D Brownian dynamics resulting from uniform

collisions by the surrounding molecules of the fluid. It is in agreement with Fig. 2b. Both results are necessitated to ensure a constant thermal bath of uniform geometry, a uniform pH and viscosity of the fluid for isotropic random-walk.

In Fig. 3, the result is a curve facing downwards. The slope of the tangent to the curve, which gives the diffusion coefficient, varies with time for the confined random walk, indicating a non-uniform diffusion, unlike isotropic random-walk where diffusion is uniform. The discrepancy is due to the restricted motions of the bio-enzymes in the cells of living organisms resulting from non-uniformity of its geometry that gives rise to non-uniform density distribution. In this case, thermal fluctuation occurs due to random collisions from the surrounding molecules of the acid fluid (DNA fluid). The condition that necessitated the result is the same in Fig. 2 except that the cells of living organisms in this case are of non-uniform geometry. Fig. 3a is consistent with Fig. 3b.

Fig. 4 is the result of an independent tracking of Brownian particles of various diameter suspended in a fluid obtained from plotting vertical column of values of MSD against time, respectively, for three bio-enzymes of different sizes in Table 6. In this case, the diffusion is constant within any time interval of motions. This is for unrestricted and isotropic diffusion. It is a three point segment graph, the fitting of which gives a straight line. The two results are in good agreement. The condition and result are the same as in Fig. 2, except that the former is a one line segment graph.

Fig.5a is the result obtained from plotting the horizontal row of values of MSD against time in Table 6. In this case, the diffusion varies within any time interval of motions. It is a partial confined random-walk associated with thermal motions of different sizes of bio-enzymes in the cells of living organisms for 1-D Brownian dynamics. The result is a zigzag line graph (Fig. 5a), and for 2-D, it is a hopping nature of diffusion (Fig, 5b). The results vary slightly due to difference in dimensions. According to Radenovic, Fig. 5b is the type of random-walk associated with bio-enzymes in the cells of living organisms in two dimensions [15].

We now propose a possible solution to the problem of thermal motion. The result of Fig. 2a for isotropic random walk proffers a solution to the problem of thermal motion due to thermal fluctuation of transcription enzymes as discussed above. In Fig. 2a, the plot of MSD versus time from Eqn. (5) $\langle (X)^2 \rangle = 2Dt$ gives a straight line, which implies uniform diffusion. Moreover, from Eqn. (7) a uniform diffusion coefficient D or diffusion implies uniform dissipative constant γ at a constant absolute temperature, T, since K_B is a constant. And, from Eqn. (9) a uniform dissipative constant γ implies a uniform number of collisions per second n between the Brownian particle and the surrounding molecules of the fluid of mass m from both left and right in one dimension, since m is a constant. Therefore, since n is uniform, it implies that the reciprocal of n is the mean time of collisions $\frac{1}{n}$ and hence the time between collisions occurs at a regular interval of $\frac{1}{n}$ for the isotropic random-walk.

In 1999, B. G. De Grooth, in Netherlands laid emphasis with respect to Eqn. (9), that dissipation would occur if the time between collisions would not be a random variable but would occur at a regular time interval of $\frac{1}{n}$. In that case, the particle would be damped and would not fluctuate, which implies that the first and second equalities of our Eqn. (5) would not be applicable. He further stated that the reason for the fluctuation-dissipation theorem is that the time between collisions is a random variable [6].The above explanation and remark emphasized the result of Fig. 2 as purely random-walk or diffusion without thermal fluctuation of the bio-enzymes. The result is necessitated by the following conditions: (a) using a constant thermal bath of uniform geometry; (b) ensuring uniform pH of the fluid by using 1-2 drops of buffer solution; (c) ensuring uniform viscosity of the fluid by using 1-2 drops of glycerol; (d) giving sufficient time for the system to attain stationary state. With the above conditions strictly observed, geneticists could possibly obtain 100% efficiency of conducting DNA test using imaging techniques without thermal fluctuations of transcription enzymes. This implies that they would have the exact imaging structure of the chromosomes of an offspring and that of the parents to be the same and equal in all respect when scanned, otherwise, there is genetic some variation. There would be no need for back up test such as blood group or genotype in the absence of the genetic traits for both species as previously done. These traits include; mental illness, exposure to excessive smoking, exposure to intensive radiations, sickle cell anemia, and albinism. They are the mutants that cause random changes in DNA by altering the physical structure of the chromosomes and hence introduce new characteristics.

Caution should be taken to ensure that the constant temperature of the thermal bath should not

exceed the survival temperature of the bio-enzymes. Otherwise, we endanger the lives of the DNA transcription enzymes.

5. Conclusion

It could now be seen that the analytical application of the 1-D Brownian dynamics to the thermal motions associated with DNA transcription enzymes evidently proffers a solution to the conduct of DNA test without thermal fluctuations of the transcription enzymes. Comparative studies of our analytical results with that of Radenovic are in good agreement, showing consistency in the two results. In proffering a solution to the problem of thermal motions in connection with bio-enzymes, we relied heavily on the condition that necessitated the elimination of perturbation due to thermal fluctuations, by adhering strictly to those conditions as per observations carefully undertaken in [15] during simulation on SPT for isotropic random-walk.

However, the non-uniformity of the pH value of the fluid, viscosity of the fluid and the thermal bath are the intrinsic factors that induce random collisions and which must be uniform for thermal fluctuations to vanish in DNA conformational dynamics.

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