MEMBRANES INTERACTIONS MEDIATED BY STICKERS: A SIMULAION STUDY

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Abstract

In this work, we investigate Moleculare dynamics simulation of Adhesion bond between receptors and ligands. We modeled the ligand-receptor pairs(Adhesion bond) as Brownian particle in viscous medium. Using the Langevin equation and employing the fourth order Runge kutta method the position of the ligand-receptor pair is determined. Based on the position's of ligand-receptor pairs the energy landscape of adhesion bond is obtained. The coupled processes (formation and breaking) of adhesion bonds are simulated by the Gillespie algorithms. These ligand-receptor interactions are modeled in the coupled chemical reactions model where breaking and rebinding of the adhesion bonds are stochastic. The Adhesion bond life time as the stochastic process that is rely on probability for bond breaking and rebinding of different realizations of the SSA are used. The stochastic relation between Nt, γ and f with $\frac{N_b}{N_t}$ was examine.

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Chapter 1

Introduction

There are many processes that (Biological)living cell perform to livelihood, maximize life processes as well as to respond changes in the environment, among several processes bond formation, particularly adhesion bond, which is non convalent bond formed between cell- cell and cell-extracellular matrix. The adhesion bond (cell)is formed and mediated by specific interaction between ligands and receptors, which is to form weak, short ranged nonconvalent bond, but that is much stronger in comparsion with nonspecific forces[17]. The adhesion bond (cell) is differ considerably from the adhesive contact in engineering systems, this is because of the nature of specific binding material between cell's surface proteins (receptors) and complementary molecules (ligands) on either cells or substrates[20]. The adhesion bond is common in a multicellular organism, where cell adhere to each other and to the extracellular matrix through a large variety of different receptor-ligand adhesion bonds[2]. Thus, the major purpose of forming adhesion bond by the Biological cells is to perform normal physiological functions such as migration, spreading, differentiation, growth and healing(repairing)[20].

The science of adhesion bond in living cell is an important phenomenon in many fields of science and technology, for-instance in biology, the main objectives are to describe the consequences of intercellular attachment on the biochemistry and function of cells, where as in physics, the objectives are to relate submicroscopic actions of bond formation and

bond break to molecular structure.

The cell adhesion bonds are characterized by the following processes: The formation of a bond is strongly influenced by the breaking of a bond, the dissociation kinetics under a mechanical force, is an intrinsic property of the ligand-receptor complex(bond). In it's natural function setting adhesion bond usually operate cooperatively with in clusters[2,1]. The clusters of adhesion bond open up the possibility for rebinding of broken bonds, which is known to be essential to achieve physiological lifetimes of adhesion clusters. These cooperative working nature of the bonds in a cluster leads to the general belief that the dissociation of a cluster is conceptually different from that of a single bond, therefore the physical description of single adhesion bonds under force now has to be extended to cluster of adhesion bonds under force. In normal life condation, fluctuations in thermal energy can break a bond occasionally and open bonds can rebind as long as other bonds are closed, thus keeping the spatial proximity required for rebinding[1]. The completely dissociated state is reached, when rebinding is impossible and then the cluster disintegrates as a whole. The single molecular pulling experiments, which is in contrast to rebinding of a broken bond where often negligible due to the elastic recoil of the transducer.

The Bell's (1978) work was a cornerstones for the development of adhesion bond (cell)in physics, his work on ligand-receptor systems investigated so far show an exponential increase of the dissociation rate with force in the limit of small forces. This concept can be viewed as a linear decrease of the free energy for dissociation, which is expected for a single sharp dissociation energy barrier along the dissociation path [3]. Thus, it is believed that pulling lowers the energy barrier of bond dissociation, that is if we apply force f on a bond, then the energy barrier is reduced by fa, where a is an interaction range of the bond[1]. Single molecule pulling experiments are deals with the extracting information associated with the kinetics and the energy landscape along the dissociation pathway of adhesion bond. The dissociation kinetics of adhesion bond is described by structured energy landscape, that is dissociation proceeds via intermediate bound states,

and then several transition states to the unbound state, which is explained by a network of transition rates (force-dependent)between the states[8]. Now-a-days there is much interest in the study of bond breaking and rebinding under force as a stochastic process. Most of the previous works are based on adhesion bond's modeled with Kramers theory that is bond breaking as thermally activated escape over a sequence of transition state barriers [1,4].

In this work, we will consider a model adhesion bonds cluster of N parallel bonds of ligand-receptor pairs between the cell and the substrate surface, where extrnal force is applied, and then the Brownian particle dynamics that subjected to a potential energy model are applied for ligand-receptor pairs. Based on this, we determine the trajectories of particle diffusion position of particle(Brownian particle) which is pairs of ligands-receptors. The free energy landscape of adhesion cluster is determine as a function of force at constant displacements. Since the free energy is a function of a single variable, many concepts in the association and dissociation kinetics of a single bond is used to study adhesion clusters[1]. Applying Gillespie algorithm, the deterministic model that has introduced in a seminal paper by Bell(1978) was extended to the stochastic modeling and also the stochastic model that depend on the binding probability between ligands and receptors on opposing surfaces as a function of contact time is considered. Here we pay more attention to generic features of the stochastic dynamics of a cluster of parallel bonds under shared constant loading and with rebinding.

The main purpose of this work is to construct an energy landscape of receptors-ligands adhesion bonds interaction by simulations. To determine the position of ligands-receptors(bond dynamic trajectories as the Brownian particle diffusion) by Simulation. And the mean life time of adhesion bond for different number(size) of adhesion bods. The dependance of mean life time on cluster size, rebinding rate constant and on external applied force will investigate by means of simulations.

Chapter 2

Brownian Dynamics of Adhesion Bond

2.1 Introduction

The aim of this section is to introduce basic concepts in cell Adhesion bond in physics and to explain the effect of external force on bonds life time. Here we consider the pairs of ligand - receptor as the Brownian particle of unit mass in $1D$ in the highly viscous medium under the influence of the external force. The dynamics of Brownian particle governed by langiven equation. His equation has two part the deterministic(conservative) part of particle dynamics and the fluctuations(dissipation) part of particle dynamics which is the effect of thermal environment and Brownian particle collision. The energy dissipation part is given by langiven equation as viscous drag force with coefficient of friction γ and the remaining fluctuation force in the form of thermal noise $\Xi(t)$. These two effects are not independent of each other since they both have the same origin which is the interaction of the Brownian particle with huge number of microscopic degree of freedom.

The convenient and parallel approach for the separation kinetics description of ligand-receptor pair is Brownian particle dynamics simulationis. Let assume that the total force

acting on pair of receptor-ligand is superpostion of the force on the intrinsic receptor-ligand bond force, that is shared external pulling force, and the Brownian particle force are along separation coordinate. The force assisted rupture process of the bound complex along the reaction coordinate x on the free energy landscape is equivalent to the escape of an overdamped particle from a kinetic trap, $U(x_i)$ with interaction range a, under a pulling force. Since the force is not directly acting on the bonded site (e.g.,ligand) in single-molecule pulling experiments, there exists a linkage connecting the bonded site to the force-acting site (e.g., probe), which is under constant pulling. Assume that the force acting on the probe is delivered to the ligand through an elastic linkage with spring constant, k_s. The overdamped particle with friction coefficient γ, is trapped in the energy well and subject to the force through the linkage, then bond under goes Brownian particle motion in the force field. Thus, relative position of the pairs along the separation coordinate x_i is determined from the over damped langiven eqaution. The ligand is under a constant pulling force, F, which is delivered through the connected spring to the bonded particle. Since the response of the probe to the thermal fluctuation kicking the ligand cannot be instant, the dynamics of the probe must be taken into account[5].

In order to investigate the bond-rupture kinetics by external pulling force: There are some conditions that have to be fulfilled for applied force, the maximum slope of interaction potential that is know as critical force has to be greater than the thermal fluctuation force and external applied force, $F_c > F > \frac{k_B T}{a}$. And for the soft linkage case, $k_s a \ll \frac{k_\beta T}{a}$, that shows a weak correlation between the ligand and probe. Where the spring delivers the external pulling completely, and the bond rupture is dominated by the ligand's Brownian particle like motion. On the other hand, the stiff linkage, $k_s a \gg F$, that results in a strong correlation between the ligand and probe. The ligand and probe can be considered as one rigid object that tries to escape from the energy well with little Brownian particle diffusivity[5]. Therefore, the effects of thermal fluctuations leads to random displacement

of the receptor-ligand bonds positions. If the response of the spring and probe(where force exerted) is instantaneous, then the ligand is assumed as under the condition of constant pulling force, F, after each time step, Δt. In this limit, the response time associated with the spring and probe is small , so comparable with the Brownian correlation time[5].

2.1.1 Forced Kramer's rate theory

The Dynamics of a particle subjected to a potential energy or Kramer's problem is a long standing problem in the area of stochastic simulation of dynamic system. He consider a particle in a deep potential well and studied the escape of the particle over a potential energy. Even though his theory does not consider the effect of linker, it enable us with insights to analyze the influences of linkage stiffness. Hence, the analytical rate constant under constant force, F, for a model potential, that has adopted for calculating the rupture rate of elastically coupled ligand and probe by overdamped Langevin dynamics [5]. Thus, kinetics of bond rupture is modeled by the escape of an overdamped particle from an energy well, where the adiabatic approximation is valid. If the potential energy $U(x)$ is non-linear one has to use numerical method to implements some Mathematical approximation to solve escape rate($MFPT$) of the particle subjected to potential energy. The survival probability $P_s(t)$ that satisfies the first order rate equation with a time dependent rate constant, $K(t)$ is given as $\frac{dP_s(t)}{dt} = -K(t)P_s(t)$. Applying some algebra, the probability distribution of the life time is given as $-\frac{dp_s(t)}{dt}$ and then the mean life time of bond is given as $\langle T\rangle = \int t(\frac{dp_t}{dt})dt$. The rate constant defined as $K = \langle\tau\rangle^{-1}$ and evaluated as $K = \frac{1}{\tau}\exp(-\beta E_a)$, where $\beta = (K_\beta T)^{-1}$ and

$$\frac{1}{\tau} = \frac{|U''(x_+)U''(x_-)|^{\frac{1}{2}}}{2\pi\gamma} \tag{2.1.1}$$

, where γ is friction coefficient of overdamped particale in the well. The positions x_- and x_+ denotes the well and saddle point, respectivly. The minimum energy which is

the activation energy required to begin the bond break-formation processes and related with the energy barrier as $E_a = U(x_+) - U(x_-) \gg K_\beta T$. When external force F_a applied, then the trapping potential becomes $V(x) = U(x) - F_a x$. Therefore, both well and saddle points are changed with externally applied force. Where, if F_a is obtained from the potential energy funcation $F_a(t) = -\frac{\partial U(x(t))}{\partial x}$ and the barrier height becomes $E_a = V[x_+] - V[x_-(t)]$. Even though E_a and τ vary with time the survival probability, $P_s(t)$, of the Adhesion bond satisfies the Brownian particle properties adjusted to the apparent potential, $V(x)$, instantaneously[5].

2.1.2 Expression of forced dissociation of adhesion bond in different regime

The single receptor-ligand adhesion bond pulling experiments are effective in extracting dynamic parameters of bond, that are to extract useful information related with the kinetics such as activation energy, interaction range, dissociation rate constant and the energy landscape along the dissociation of sequence of reactions [7]. The primary role of external force applications (pulling) in the dissociation kinetics is to lower the energy barrier E_a, likewise, catalyst does in chemical reaction process. As shown in the Fig.2.1

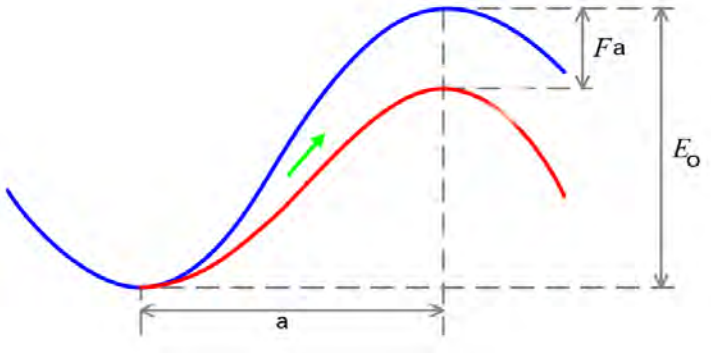

Figure 2.1: Schematic representation of the energy landscape for receptor-ligand interaction. The binding state at left is separated by a transition state barrier from the broken state. The transition state barrier is characterized by the height E_o and by the interaction range a to the closed state. An applied force tends to tilt the energy landscape resulting in a lower barrier height [20].

As stated in the seminal work by Bell, it is assume that the metastable potential well is deep and then the force-dependent energy barrier is given as $E_a(F) = E_o - Fa$, where E_o represents the intrinsic activation energy and a is interaction distance between the potential well and the barrier along the interaction coordinate. For a long time, Bells expression is taken as a phenomenological theory that is convenient to extract the intrinsic dissociation rate constant K_{off} and interaction range, a, from pulling experiments. However, another work by Garg assume that bond rupture occurs when the metastable well becomes very shallow; in his case a cubic function is appropriate to approximate the potential surface in the vicinity of the critical force (F_c) and $E_a = E_o(1 - \frac{F}{F_c})^{\frac{3}{2}}$.

Much work have been carried out in this area recently with a fitting parameter ν, and there are proposed approachs to extract kinetics parameters such as K_o, a and E_o. In this approach a escape rate under force F is given as $K(F,\nu) = \frac{1}{\tau(F,\nu)} * \exp^{[-\beta E_a(F,\nu)]}$, where the activation energy is $E_a(F,\nu) = E_o(1-\nu\frac{Fa}{E_o})^{\frac{1}{\nu}}$ and $\frac{1}{\tau(F,\nu)} = K_o(1-\nu\frac{Fa}{E_o})^{\frac{1}{\nu}-1} * \exp(\beta E_a)$. Bell's expression is valid for $\nu = 1$ or as E_a goes to infinite, while the Garg form is obtained for $\nu = \frac{3}{2}$. It is believed that Garg expression form is more general than Bell. However, both Bells and the Garg's expression form are valid in different regimes for single-well potential that correspond to two asymptotic limits, which are Slow (weak)pulling regimes $\frac{F}{F_c} \ll 1$ and fast(strong)pulling regimes $1 - \frac{F}{F_c} \ll 1$, respectively. The Bell's and Garg's expression form are valid at the asymptotic limit, and which is also in agreement with the Kramers rate theory. There are three parameters, by which the free energy landscape is related with $U(x)$ and characterized by: interaction range that represents the distance between the metastable well x^o_- and the energy barrier x^o_+, the intrinsic energy barrier $E_a = U(x^o_+) - U(x^o_-)$ and the maximum slope of the trapping potential, which is called critical force, i.e $F_c = U'_o(x_c)$. The environmental factories like thermal fluctuations in some conditions can break adhesion bond. If the intrinsic energy barrier, E_a, is greater than thermal energy, $E_a \gg K_\beta T$, then the dissociation rate of the adhesion bond is define in the frame of the Kramers theory as $K_{off} = \frac{1}{\tau}\exp^{-\beta E_a}$,

with $\tau = \frac{2\pi\gamma}{\omega_- \omega_+}$. Here $\omega_\pm = [\frac{\mp U''(x_\pm)}{M})]^{\frac{1}{2}}$. This thermally activated dissociation (escape) represents the kinetic limit. Where as, when the external applied force, F_a, is greater then critical force, F_c, then the binding force can be overcomed without any help of thermal fluctuations. Briefly the dissociation is dominated by external pulling; which is know as mechanical limit. If the externally applied force is between , 0, and maximum force, F_c, then it alters the effective trapping potential and the kinetic parameters. Therefore, the effective trapping potential becomes $V(x) = U(x) - F_a x$, the barrier height becomes, $E_a(F_a) = V[x_+(F_a)] - V[x_-(F_a)]$, τ bond life time becomes $\tau(F_a)$, and the rate constant, $K_{off} = \frac{1}{\tau} \exp^{-\beta E_a}$. Hence, the energy barrier is lowered and the bond lifetime is reduced with increasing external applied force F_a.

2.2 Analytical expressions for the bond lifetime under constant force in the asymptotic limits

The asymptotic limits expresstion for the bond lifetime under applied constant external force is given for the regimes : $\frac{F}{F_c} \ll 1$ and $1 - \frac{F}{F_c} \ll 1$. If the limit regime $\frac{F}{F_c} \ll 1$ is considered, then the ligand-receptor positions $x(_\pm)$ is close to $x^0(_\pm)$, and then the trapping potential near $x^0(_\pm)$ approximated by Tayler series(by taking only up to second order)

$$U(x_\pm) = U(x^o_\pm) + \frac{1}{2} U''(x^o_\pm)(x_\pm - x^o_\pm)^2 \tag{2.2.1}$$

By putting $U'(x^o_\pm) = 0$. As the consequence the new positions of well and saddle point are determined by

$$x(_\pm)(F) = x^0(_\pm) + \frac{F}{U''(x^0_\pm)} \tag{2.2.2}$$

Then the dissociation rate is, $K_{off} = K_o \exp(\beta F a)$; this is the well known Bells expression. The basic assumption in the preceding derivation is the well and barrier are slightly shifted away from the original positions. Consequently, Bells expression is valid when the pulling force is weak[7].

When the limit regime $1 - \frac{F}{F_c} \ll 1$ is considered, then the ligand-receptor position $x(_\pm)$ is in the proximity of x_c; therefore, the bond breaking rate is found by expanding $U(x_\pm)$ near x_c. By setting $U''(x_c) = 0$ and $U'(x_c) = F_c$. Then we have

$$U'(x_\pm) = F_c + \frac{U'''(x_c)(x_\pm - x_c)^2}{2!}, \tag{2.2.3}$$

where x is close to x_c. If $U'''(x_c) = 0$, then we have to expand to higher order

$$U'(x_\pm) = F_c + \frac{U^{n+1}(x_c)(x_\pm - x_c)^n}{n!} \tag{2.2.4}$$

Here $n > 2$ is suitable because $U'(x)$ has maximum value at x_c. This is the similar to following approximation:

$$U \sim \frac{F_c b}{2}[y - \frac{y^{n+1}}{n+1}] + U(x_c), \tag{2.2.5}$$

where $y = \frac{x - x_c}{b/2}$ and $b = 2[\frac{-n!F_c}{U^{n+1}(x_c)}]$ is the characteristic length of U in this regime[7]. And the location of energy well and energy barrier for the potential energy $V = U - Fx$ is

$$x_\pm = \pm\frac{b}{2}(1 - \frac{F}{F_c})^{\frac{1}{n}} + x_c \tag{2.2.6}$$

The energy barrier height and the intrinsic time scale are expressed as

$$E_a = V(x_+) - V(x_-) = E_a^*(1 - \frac{F}{F_c})^{n - \frac{1}{n}} \tag{2.2.7}$$

$$\frac{1}{\tau_{(t)}} = \frac{2\pi m\gamma}{|U''(x_+)U''(x_-)|^{\frac{1}{2}}} = \frac{1}{\tau^*}(1 - \frac{F}{F_c})^{n - \frac{1}{n}}, \tag{2.2.8}$$

where $E_a^* = \frac{n}{n+1}bF_c$, $\frac{1}{\tau^*} = \frac{(n+1)\beta E_a^*}{[\pi(\frac{b^2}{D})]}$ and $K(F) = \frac{1}{\tau^*(F)}\exp^{[-\beta E_a(F)]}$. Thus, the Bell's expression drawback is it unable to reveal much information about crtical force, eventhough useful to extract the intrinsic properties such as K_{off} and a. Hence, we are most interested in the regime is $1 - \frac{F}{F_c} \ll 1$, where the free energy landscape is altered, and the apparent energy barrier is proportional to $(1 - \frac{F}{F_c})^{\frac{3}{2}}$. This implies that we can got some clue about the F_c from strong pulling experiments[7].

2.2.1 The Free energy landscape of Adhesion Bonds Clusters

Let us now briefly present how the free energy landscape of the adhesion bond is obtain from force-displacement relation, that is useful in studying the dissociation of the adhesion bond [1]. Here the adhesion bond cluster of, N, parallel ligand-receptor pairs between the cell and the substrate surfaces(EMS)is considered. The dynamics of a ligand-receptor pair is modeled as an overdamped(high fiction) particle i at position x_i in a interaction potential $U_o(x_i)$, that represents ligand-receptor interaction, plus the effect of the elastic linker that is given as $\frac{1}{2}k_s(X_o - x_i)^2$. When the substrate is moved to X_o due to external applied force each particle is pulled by force, $F = \sum_i k_s(X_o - x_i)$.

The theoretical study for the dynamics of adhesion bond cluster will be more simplified if the average ligand - receptor distance $\bar{x} = \frac{\sum x_i}{N}$ is the only slow variable. And then we can apply the langiven equation for this slow variable $\bar{x}$

$$(N\gamma)\frac{d\bar{x}}{dt} = -\frac{d\omega}{d\bar{x}} + \Xi(t) \tag{2.2.9}$$

, where ω is effective potential that is function of $\bar{x}$, the fluctuating term is is know as noise. Such partial differential equation that contains randomly fluctuating term is classified as stochastic partial differential equation, and the corresponding process is stochastic process, which is a process that evolves probabilistic of some event with time. In stochastic process the evolution of the system with time may have many possibilities for known(fixed) initial condation. Thus, probability theory is the main tool in dealing with stochastic phenomena. The ideal noise(white noise) is a noise which fluctuates very rapidly so that $\langle \Xi(t) \rangle$ and $\langle \Xi(t') \rangle$ can be statistically independent event for very small time interval$|t-t'|$,i.e. means no correlation time. The mean value is $\langle \Xi(t) \rangle = 0$, that is average over independent realisation of random process and the variance $\langle \Xi(t)\Xi(t') \rangle = 2N\gamma K_\beta T\delta(t - t')$. The assumption of no correlation time is a very powerful, because it simplifies the problem, and so that we can treat the system as Markovin process. By Markovin we mean that a process which it's future depend only on the present. However, the time scale of

fluctuation might not be much shorter than that of the Brownian particle. Consider the limit of large, γ, that results in a very short relaxation time τ, this intern corresponds to the adiabatic elimination of fast variable velocity, by putting the $\ddot{x} = 0$ in the equation of motion given by Langevin, that is Eq. 2.2.9. Thus, for colored noise we have to use finite correlation time τ. If the Eq. 2.2.9 holds, then the adhesion bonds cluster treated as a particle under effective potential $\omega(\bar{x})$. Before look for the effective potential, let consider the cluster of adhesion bond under given (N, X_o, T) at thermal equilibrium. Here force is given as function of displacements $F(X_o)$, which is an equation of state. The force, $F(X_o)$, is an ensemble average that associated with a microscopic state, $F\{x_i, X_o\}$, over equilibrium distribution x_i at given (N, X_o, T). This is suitable for describing the thermodynamics of $N \gg 1$ adhesion bond clusters and to obtain the effective potential. The effective potential and force that required to maintain a fixed displacements can determine from the partition function of $N \gg 1$ parallel adhesion bonds[1].

$$Q = \int \cdots \int \exp^{-\beta H} \, dx_1 .... dx_N, \tag{2.2.10}$$

where Hamiltonian is given as $H = \sum_{i=1}^{N} [U_o(x_i) + \frac{1}{2} k_s (X_o - x_i)^2] = \sum_{i=1}^{N} H_i$. Then Eq. 2.2.10 becomes

$$Q(X_o) = [\int \exp^{(-\beta H_i)} \, dx_i] = (Q_1)^N \tag{2.2.11}$$

$$\omega_o(X_o) = -K_\beta T ln^{Q(X_o)} = -K_\beta T ln^{Q_1^N} = -N K_\beta T ln^{Q_1} \tag{2.2.12}$$

Then the froce is determine as follow from the preceding equation

$$F(X_o) = -\frac{\partial}{\partial X_o}[\omega_o(X_o)] = N K_\beta T (\frac{\partial}{\partial X_o} ln^{Q_1})_{N,T} \tag{2.2.13}$$

Since the two potential minimal, that associated with bound state at x_- and free state at x_+ is used for determine the force $U_o'(x_\pm) = k_s(X_O - x\pm)$. Then the single particle partition function is given by saddle-point approximation.

$$\int_0^\infty \exp(-\beta H_i) dx_i = \sqrt{\frac{2\pi k_\beta T}{U''(x_+) + k_s}} (1 + \frac{k_{on}(X_o)}{k_{off}(X_o)}) \exp(-\beta [U_o(x_+) + \frac{k_s}{2}(x_+ - X_o)^2)]) \tag{2.2.14}$$

where

$$\frac{K_{on}(X_o)}{K_{off}(X_o)} = \sqrt{\frac{U_o''(x_+)+k_s}{U_o''(x_-)+k_s}}\exp(-\beta[U_o(x_-)-U_o(x_+)+\frac{k_s}{2}(X_o-x_-)^2-\frac{k_s}{2}(X_o-x_+)^2]) \tag{2.2.15}$$

The effective potential is given by

$$\omega_o(X_o) = N[U_o(x_+)+\frac{k_s}{2}(x_+-X_o)^2]-Nk_\beta T[ln\sqrt{\frac{2\pi k_\beta T}{U_o''(x_+)+k_s}}+ln(1+\frac{k_{on}(X_o)}{k_{off}(X_o)})] \tag{2.2.16}$$

When the pulling is such that X_o is located out side of interaction range a of a bond, the free state $x_+ \approx X_o$ the potential energy with free state given as $U(X_o = x_+) \approx$ constant. If Eq. 2.2.16 hold the force at X_o in condition where $x_+ \approx X_o$ and $U_o(x_+) \approx U_o(X_o)$ constant.

$$F(X_o) = -\frac{\partial \omega_o}{\partial X_o} = \frac{NK_\beta T}{(1+\frac{k_{off}}{k_{on}})}[\frac{k_s}{K_\beta T}(x_- - x_o) - \frac{k_s U_o''(x_-)}{2(U_o''(x_-)+k_s)^2}] \tag{2.2.17}$$

The Eq. 2.2.17 implies that force at given X_o is shared by closed bonds. For the cluster of adhesion bond, force-displacement relation is given as

$$F(X_o) = \frac{N}{(1+\frac{k_{off}}{k_{on}})}k_s(x_- - X_o) \tag{2.2.18}$$

a number of closed bonds in equilibrium given as $N_b = \frac{N}{(1+\frac{k_{off}}{k_{on}})}$ [1] .

2.2.2 Lifetime of adhesion bonds cluster under constant force

As a results of fast fluctuates in the adhesion bond cluster at fixed substrate position, X_o around it's ensemble average $\bar{x}$ is given as

$$\bar{x} = \frac{x_- + X_o\frac{k_{off}}{k_{on}}}{1+\frac{k_{off}}{k_{on}}} = \frac{\langle N_b\rangle}{N}x_- + \frac{N-\langle N_b\rangle}{N}x_+, \tag{2.2.19}$$

Where $\langle N_b\rangle$ is ensemble average of the number of closed bonds in the cluster and the position X_o expressed as funcation of $\bar{x}$, i,e $X_o = X_o(\bar{x})$. The constant force free energy of the adhesion bond cluster is related by expression $\omega(\bar{x}) = \omega_o[X_o(\bar{x})] - F\bar{x}$. When the

potential energy, $\omega(\bar{x})$ has metastable well, then dissociation of adhesion bond cluster with N parallel bonds becomes equivalent to forced escape of a single bond[1].

Since both the barrier height, E_a and the rate constant, τ of the adhesion bond cluster depend on F, and for the forces, $F > F_c$ there would be no solution for $x_\pm$ because the metastable well and the barrier do not exist. The rate equation that describes the kinetics of the ensemble average of $\langle N_b \rangle$ in Eq. 2.2.19 can be reorganized into a deterministic equation for $\bar{x}$. In other word, this equation is simply Eq.2.2.9 without the noise term. Therefore, the infinite lifetime for force below Bell's critical force predicted by the rate equation simply indicates the appearance of a free energy barrier in the dissociation pathway in $\omega(\bar{x})$. Bonds with constant rebinding rate were considered, but in principle we can applies to bonds with any force dependent rebinding rate[1].

2.2.3 Single bond mechanics

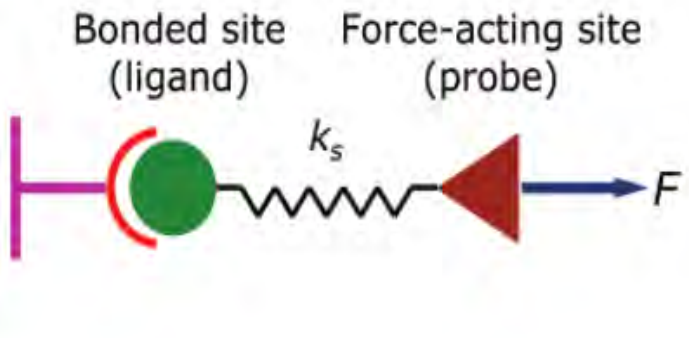

Figure 2.2: Schematic representation of single bond pulling experiment. Force act on ligand through elastic linkage[5]

The response of adhesion bond is different, even for the same external force, this is due to the stochastic nature of the system. However, upon observing many of the responses, the general distribution of the dynamic properties can be found[9]. The environment factors such as thermal noise is an integral part of the bond separation process. Therefore, the response of the adhesion bond to an external applied force can only be described

in probabilistic terms. Let's assume the instantaneous configuration of the bond fully identified by a single variable x, which used as the interaction coordinate. And the adhesion bond under this condation adopt to the configurations confined to $1D$ free energy landscape $U(\mathrm{x}, t)$, which is the sum of the bond natural free energy and the external force acting in the direction of x. Now we consider an ensemble of identical bonds that are simultaneously subjected to the same external force, responding independently of each other. Therefore, we take this concepts as the probability distribution of the adhesion bond configurations that is diffusing on the free energy landscape, $U(\mathrm{x}, t)$, governed by the generalized diffusion equation.

The fraction of the bonds in the ensemble that remain intact (not changed) at time t is the survival probability of the bond. To describe force-induced bond rupture, a common starting point is the first-order rate equation governing the evolution of the survival probability (the first order kinetics is rare character of the rupture events). If no force applied, the rate for barrier crossing k_o depends on barrier height, E_b, given as $k_o = \exp\left(\frac{\frac{E_b}{K_\beta T}}{t_D}\right)$, where t_D is attempt time which is for adhesion bonds on the scale of nanoseconds. According to Kramers theory, force applied to the bond tilts the energy landscape through an additional term $-Fx_b$, where x_b is the distance between bound state and barrier along the linear reaction coordinate. By setting an internal force scale $F_b = \frac{K_\beta T}{x_b}$ and the transition rate give as $K(t) = k_o \exp\left(\frac{F(t)}{F_b}\right)$ as has done by Bell. In the adiabatic approximation, one assumes that the escape process takes no time. Thus, the probability to rupture between t and $t + dt$ is given as $P(t)dt = K(t)S(t)dt$, where $K(t)$ is instantaneous rupture rate and $S(t)$ is the cumulative probability for survival up to time t[16]. This quantitiy satisfies the relation:

$$S(t + dt) = S(t)(1 - K(t)dt) \Rightarrow S(t) = \exp\left(-\int K(t)dt\right) \tag{2.2.20}$$

In the adiabatic approximation bond lifetime is expressed as

$$\tau = \int_0^\infty P(t)tdt \tag{2.2.21}$$

From all the cases the simplest one is constant loading case, where $K(t)$ is independent of time, $S(t) = \exp(-Kt)$, $P(t) = K\exp(-Kt)$ and $\tau = \frac{1}{K}$. This is nothing else than the classical Poisson process, or, in the language of chemical kinetics, first order dissociation[16]. The time dependence of the rupture rate in this equation can be calculated by treating bond rupture under force as a diffusive barrier crossing a generalized version of a classical problem studied by Kramers[9].

2.3 Master equation for One-step processes

One step processes, which is a continuous time Markov process whose range consists of integers n, where $n \geq 0$ and whose transition matrix, W, permits only jumps between adjacent site[14].

$$W_{nn'} = r_{n'}\delta_{n,n'-1} + g_{n'}\delta_{n,n'+1} \tag{2.3.1}$$

,where $n \neq n'$. The single receptor ligand adhesion bonds have finite lifetimes, so that biological systems can dynamically react to changes in their environment. In cell adhesion, adhesion bonds commonly act cooperatively in adhesion bond. Analytical solutions for the appropriate one-step master equation are presented for special cases, while the general case is treated with exact stochastic simulations. Here we use the stochastic version of the Bell model to study the case of constant shared loading. And in contrast to applications to specific experiments, the focus is given to the generic features of the stochastic dynamics of a cluster of parallel adhesion bond (all bonds are loaded simultaneously (parallel loading), that can be realized experimentally by moving the transducer perpendicular to the substrate) under shared constant loading and with rebinding.

There are several advantages of stochastic model over deterministic model:

i).The only means to treat the experimental situation, that rebinding becomes impossible once the completely dissociated state has been reached is the stochastic model.

ii).The fluctuations and nonlinear effects, which are important for small adhesion

clusters can be seen in the stochastic model.

iii).Using the master equations, the stochastic model allows us to derive analytical solutions for cluster lifetime as a function of cluster size, rebinding rate constant and force, which are very helpful in evaluating adhesion experiments [10].

vi) The deterministic model is based on the differential equation, while Stochastic model is based on Simulation.

The thermally activated escape over a transition state barrier is consider for the infinite transition state barrier, and then it leads to Bell equation for a single bond dissociation rate as a function of force, $K_{off} = k_o \exp(\frac{F}{F_b})$. Here the force scale $F_b = \frac{K_\beta T}{X_b}$ is set by thermal energy $K_\beta T$ and the distance X_b is interaction range. The dissociation rate (k_{off}) depends mainly on the internal structure of a bond and the association rate (k_{on}) formation of bond, which is not easy to determine experimentally for cell adhesion, when the interacting bonds are anchored to opposing surfaces. Here the focus is on the generic features of cluster stability and the assumption is a force independent constant. Defining dimensionless quantity:dimensionless time $\tau = k_o t$, dimensionless force $f = \frac{F}{F_b}$, dimensionless rebinding rate constant $\gamma = \frac{K_{on}}{K_o}$ and dimensionless single adhesion bond dissociation rate is $\frac{K_{off}}{K_o} = \exp(f)$. Consider the situation in which N_t receptor - ligand pair are arranged in parallel along cell- substrate interface. Since all bonds are identical, so that the state of the adhesion bonds cluster is completely characterized by the number of closed bond n. If the cluster consists of constant number of N_t bonds that initially all are closed and then under go breaking and rebinding according to the appropriate rates. At time t, there are n closed and $N_t - n$ open bonds, i.e the dynamic variable n range from, 0 , to completely unbounded state to completely, N_t , bounded state. Since bond rupture is a discrete process, the stochastic dynamics of the bond cluster can be described by the one-step master equation [10].

$$\frac{dP_n}{d\tau} = r_{n+1}P_{n+1} + g_{n-1}P_{n-1} - [r_n + g_n]P_n \tag{2.3.2}$$

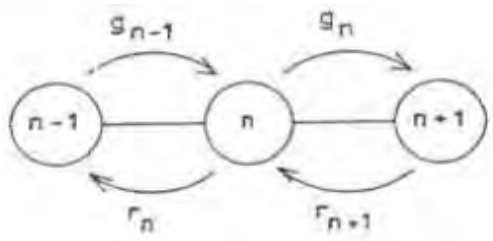

Figure 2.3: The figure depict to visualize a one step process of ME [12]

, where $P_n(\tau)$ is the probability that n bonds are closed at time τ, and diagonal elements are, ofcourse, $W_{nn'} = -(r_n + g_n)$. It is a system of coupled linear differential equation for state probability function $P_n(\tau)$. Here the r_n and g_n are the reverse and forward rates between the possible states n $(0 \leq n \leq N_t)$. The Master equation for such processes when r_n is the probability per unit time for a jump from state n to state $n-1$ and g_n is the probability per unit time for a jump from n to $n+1$ are clear in figure [2.3] [12]. These association and dissociation rates of single adhesion bonds are define as following, respectively[10].

$$g_n = g(n) = \gamma(N_t - n); r_n = r(n) = n * \exp(\frac{f}{n}) \tag{2.3.3}$$

The rate of transition of any one of the bond in the cluster is given by single bond rate times the number of candidate for respective transition and the transition of single bond also causes change in the state of the cluster. Our model has three parameters namely, cluster size N_t, rebinding rate constant, γ, and force f. Since $n \geq 0$ should be guaranteed at any time, r_o has to be set for $f > 0$, Eq. 2.3.3 besides it's definitions it implies $g_o > 0$, that is, after rupture of the last closed bond new bonds are allowed to form. This corresponds to a reflecting boundary of the master equation at $n = 0$. The rebinding of the completely dissociated state is usually prevented by elastic recoil of the transducer. Then we set $g_o = 0$ to model an absorbing boundary at $n = 0$. Due to the values given for r_o and g_o not follow the general form given in Eq. 2.3.3 the boundary at $n = 0$ is an artificial boundary. Concerning the upper end of the set of states at $n = N_t$, and from $g_{N_t} = 0$ represents a reflecting boundary that guarantees $n \leq N_t$ .Thus, the upper

boundary is a natural boundary of the master equation. A quantity of large interest is the average number of closed adhesion bonds

$$N(\tau) = \langle n \rangle = \sum_{\mu} n P_n(\tau) \tag{2.3.4}$$

From the master equation one can derive

$$\frac{dN}{d\tau} = \sum_{n=0}^{N_t} n \frac{dP_n}{d\tau} = \langle r(n) \rangle + \langle g(n) \rangle \tag{2.3.5}$$

If both $r(n)$ and $g(n)$ were linear function in n then equation(2.3.5) become an ordinary differential equation for N. Thus, this leads to the study of deterministic equation

$$\frac{dN}{d\tau} = -r(\langle n \rangle) + g(\langle n \rangle) = -n * \exp(f/n) + \gamma(N_t - n) \tag{2.3.6}$$

as has done by bell. For constant shared loading it also holds for non-linear case. This mean field description for N replace average over ensemble of stochastic adhesion cluster trajectories by single determinstic trajectory of representative adhesion cluster. It is exact for linear transition rate. This shows generic feature of our model, but the deterministic equation of the above is deviate from the average number of closed bond obtained from the solution of master equation. It is important to note that Eq. 2.3.3 for $f > 0$ the reverse rate $r(n)$ is nonlinear in n and the average in Eq. 2.3.5. can not be taken[10].

2.3.1 Numerical solution of master equation

As externally applied force increases the energy barrier for adhesion bond dissociated decreases, as results the adhesion bonds break and a number of closed bonds decreases. This further increases the dissociation rate and the fluctuations tend to change the time point of rupture than typical shape of the decay curve. However, if the cluster size increased the fluctuation become less and the rupture event are concentrated around the rupture of the deterministic cluster.

The master equation enable us to formulate exact evolution of a system through time mainly for simple cases. However, it is of little practical use when there are many reactions or non-linear reactions[13]. Thus, master equation can be solved numerically by

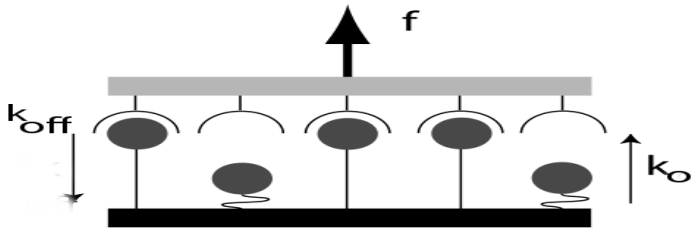

Figure 2.4: The figure depict schematic representation of our stochastic model for an adhesion cluster under shared force: in this case, there are $N_t = 5$ receptor-ligand pairs, of which $n = 3$ are closed and equally share the dimensionless force f. Single closed bonds rupture with dissociation rate $k_{off} = k_0 \exp(\frac{f}{n})$ and single open bonds rebind with force-independent association rate k_{on}. Our model has three parameters: cluster size N_t, dimensionless rebinding rate $\gamma = \frac{K_{on}}{k_o}$ and dimensionless force f [16].

Monte Carlo method for each set of parameter values N_t, γ and f. That is generating a large number of trajectories with the help of the Gillespie algorithm which leads to exact stochastic simulations. A better approach is rather than solving for all possible trajectories by the master equation, a particular evolution of a system is simulated using a stochastic algorithm developed by Gillespie, that is in order in the sense that one simulation is a particular realisation of a random evolution trajectory governed by the master equation[13]. Hence, by taking the average for given time over the different simulation trajectories we can get the probability distributions $P_n(\tau)_{n=0}^{N_t}$. By studying the single simulations trajectors that is their specific feature expected to be characteristic of experimental trajectors. Here we use the Gillespie algorithm that developed for simulation of the stochastic dynamics of coupled chemical reactions. Therefore, we can apply to case of rupture and rebinding of bonds which corresponds to two different species of molecules and the transition between these two species, that is rupture and rebinding, correspond to chemical reactions. The algorithm is efficient because it generates jumps between subsequent reactions rather than discretizing time in small steps[10].

The basic quantity of the Gillespie algorithm is the probability $P(\mu, \tau/\tau_o, x)$ that the next reaction occurs in the time interval $[\tau_o + \tau, \tau_o + \tau + d\tau]$. And the type of reaction is μ under the condition that at time τ_o the system is in state x. In our case, μ (type of

reaction) have two values corresponding to rupture and rebinding, and let the state of the system is completely described by the number of closed bonds n. Since the adhesion bonds breaking and rebinding rates from Eq. 2.3.3 are constant between subsequent events, P does not depend on absolute time τ_o. In fact it is given as

$$P(\mu, \tau/\tau_o, x) = P(\mu, \tau/n) = P_o(\tau/n)a_\mu \tag{2.3.7}$$

Where P_o is the probability that no reaction occurs in the time interval $[0, \tau]$ and a_μ is the reaction rate for reaction μ. Since P_0 satisfies the differential equation

$$\frac{dP_o}{d\tau} = -(\sum_\mu a_\mu)P_o \tag{2.3.8}$$

Then it is solved as

$$\frac{dP_o}{P_o} = -(\sum_\mu a_\mu)d\tau \Rightarrow \int \frac{dP_o}{P_o} = \int -(\sum_\mu a_\mu)d\tau \tag{2.3.9}$$

From the initial condition $P_0(0) = 1$

$$ln(P_o) = -(\sum_\mu a_\mu)\tau \Rightarrow P_o(\tau/n) = \exp(-(\sum_\mu a_\mu)\tau) \tag{2.3.10}$$

Thus, probability satisfies $P(\mu, \tau/n)$ a normalisation conditions, and that can be proved by integrating over time and summing over reactions.

$$\int_0^\infty P(\mu, \tau/n)d\tau = \int_0^\infty \exp(-\sum a_\mu \tau)d\tau \tag{2.3.11}$$

The algorithm generates trajectories that are subsequent reactions are separated by the following rule: Where there is no other reactions, the probability for a reaction μ in the time interval $[\tau, \tau + d\tau]$ is given by

$$P_\mu(\tau) = a_\mu \exp(-a_\mu \tau)d\tau \tag{2.3.12}$$

Then integrating the probability

$$F_\mu(\tau) = \int_0^\tau P_\mu(\tau')d\tau' = 1 - \exp(-a_\mu \tau_\mu) \tag{2.3.13}$$

which is the probability for a reaction occuring until time τ. It is increasing monotonically from zero to unity,i,e it can be inverted. To generate a random variable τ_μ which is distributed according to Eq. 2.3.11, by generates a random number ξ that is uniformly distributed over the interval $[0, 1]$ and then inserts it into the formula $\tau_\mu = \frac{ln(\xi)}{a_\mu}$. If this is done for each type of reaction, then we set times τ_μ. The time for the next reaction is then chosen as the smallest of τ_μ, that is $\tau = min_\mu(\tau_\mu)$. The above rules generates trajectories with the correct distribution of times and types of subsequent reactions. Using the forward and reverse rates for rebinding and unbinding of adhesion bonds in the cluster, the random times are determined as follow:

$$\tau_f = \frac{-ln(\xi)}{\gamma(N_t - n)} \tag{2.3.14}$$

and

$$\tau_r = \frac{-ln(\xi)}{n * \exp(\frac{f}{n})} \tag{2.3.15}$$

This approach is exact in the sense that the only sources of inaccuracy lie in the choice of the random number generator and the number of trajectories used for looking probability distribution[10].

Chapter 3

Stochastic Energetic for Simulation

In order to compare the theoretical work of the adhesion bond with the Langevin dynamics simulation. The Langevin dynamics simulation is carried out for N parallel ligands-receptors pair adhesion bonds for a given model interaction potential energy.

$$U_o(x) = \frac{E_a^o}{2}[1 - cos\pi(\frac{x}{a})], 0 \leq x \leq a \tag{3.0.1}$$

$$U_o(x) = E_a^o, a < x \tag{3.0.2}$$

Using the given model interaction potential energy the force displacement-relation $F(X_o)$ can be studied by the numerical simulation. The result is in good agreement with theoretical study as stated in Eq. 2.2.18, from which we can see crtical force F_c and the location X_o corresponds to crtical force. Where the crtical force is maximum of the potential energy slope. On the parallel arranged adhesion bonds force is applied and where the soft transducer are our special interest. In which the applied force is shared equally between the closed bonds which reveals the real cooperativity nature of the adhesion bonds[16]. Understanding the conformational(symmetrical disposition) dynamics of ligands-receptors intraction requires a detailed knowledge of the structure of their potential energy landscapes. Because of a large number of the particles, and the various kinds of interactions that determine the energy of a particular structure, such knowledge cannot be obtained by direct calculation. Therefore, there is a great need of simulations

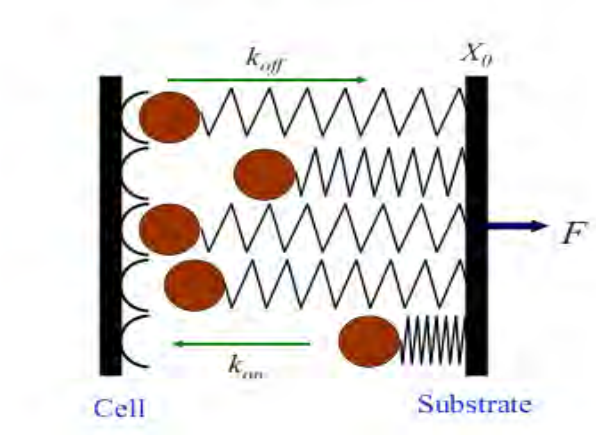

Figure 3.1: The schematics representation of an adhesion bonds. The receptors are fixed on the cell surfaces and ligands are connected to a substrates surface by springs. External force F is applied to substrate surface[1]

methods that give direct access to the potential energy surface even it has a very specific structure.

3.1 Langevin dynamics application for Brownian particle model

3.1.1 Algorithm for the simulations

We can determine the position of the particle (ligand-receptor pair/ Brownian particle) using the Langevin equation (LE) by appling Runge Kutta fourth order method. Since this method is applied for ordinary differential equation which required to be specified intial conditions and has to be linear. For the given model potential the langiven equation is used to determine interactions forces among the particle, and the thermal fluctuations force is model as the gaussian distribution. Then we can apply the RKM as follow,

$$N\gamma\frac{d\bar{x}}{dt} = f(x,t) \tag{3.1.1}$$

by $f(x,t) = \frac{dU_o(x)}{d\bar{x}}$ where, $U_0(x)$ is given in Eq. 3.1.1 and 3.1.2. Then it becomes,

$$\frac{dU_o(x)}{d\bar{x}} = \frac{\pi}{a} * \frac{E_a^o}{2}\sin\pi(\frac{x}{a}) = f(x,t) \tag{3.1.2}$$

$$(N\gamma)\frac{d\bar{x}}{dt} = f(x,t) = 0 \tag{3.1.3}$$

where, $0 \leq x \leq a$ for Eq. 3.2.2 and $a < x$ for Eq. 3.2.3.

Intial conditions that we have to specifies first is t_n and X_n to look for next points X_{n+1} at t_{n+h}. And using the weighted average of an approximated values for $f(x_n, t_n)$ at a number of time within interval $[t_n, t_n + h]$. The t value is iterated by adding a fixed step size h for each iteration and the x value is looked based on the weighted average of k_1, k_2, k_3 and k_4. Thus, each of the k_i value gives us an estimate of the size of x jump made by the actual soluation accross the whole width of the interval. That are given as follow:

$$x_{n+1} = x_n + \frac{1}{6}(k_1 + 2k_2 + 2k_3 + k_4) \tag{3.1.4}$$

where, $k_1 = hf(t_n, x_n) = h\frac{dU_o(x)}{d\bar{x}}$

$k_2 = hf(t_n + h/2, x_n + \frac{k_1}{2}) = h\frac{dU_o(x)}{d\bar{x}}$

$k_3 = hf(t_n + h/2, x_n + \frac{k_2}{2}) = h\frac{dU_o(x)}{d\bar{x}}$

$k_4 = hf(t_n + h, x_n + k_3) = h\frac{dU_o(x)}{d\bar{x}}$

In short on the flow chart can state as follow:

For a - lower limit and b - upper limit

$t_i = a + i * h$, where $i = 0, 1, 2, 3...$

$h = \frac{b-a}{n}$, where $n = 1, 2, 3, ...$ and $x(a) = x_0$ and $x(b) = x_n$

There are m times of iteration for each step, where m is integer.

Then the final position is given in Eq. 3.2.4.

3.1.2 Simulation procedure

Since we use langiven dynamics for our simulation purpose, the first thing that we do is deriving the interactions force from the given model potential energy and determining(modeling) the thermal fluctuations force. Then by setting the limit values for interactions potential range, we tried to see how the position of the ligands-receptors pairs are changed through time as explained in the preceding section. We see how the ligands-receptors postision, x_i, are changes (diffuses) for given values of substrate postion, X_o which itself is changing continuously by adding fixed value for every step. The monte carlo time step update it value by adding a fixed value ,i.e dt. Then using the particle postision we see the energy landscape of the interaction potential of ligands-receptors adhesion bond.

One dimensional adhesion bonds are ideally suited to this purpose for dual reasons that are for it's microscopic nature of ligands-receptors adhesion bonds and short life time of the bonds (computation times are shorter too) than other dimension case.

In our simulation the ligands are constructed from elastics thus, elastics potential interactions also considered. However, the elastics nature is not influence the free fluctuations of particles. Since we are investigating adiabatic approximation conditions, the initial condition may play an important role. According to our setup, a adhesion bonds of size N is initially placed in a state in which the number of particle(ligands-receptors pairs) are arranged parallel with intraction potential, thermal force and under external applied force as can be seen from figure 3.1. The above processes are repeat as long as the ligands-receptors are within the interaction range.

3.2 Stochastic simulation of adhesion bonds breaking and rebinding Algorithm

Here we consider a coupled processes(reactions) namely breaking and rebinding of Adhesion bonds that are stochastic processes; each processes has different rate and given as

$$N_t \xrightarrow{r_n} N_t - 1; N_t - 1 \xrightarrow{g_n} N_t \tag{3.2.1}$$

where, for $t = 0$, the total number of bonds are $N(t = 0) = N_t$, where as for $t > 0$ we can state the number of adhesion bonds N_t $(t > 0)$ in the system is equal to N_t with the probability $P_n(t)$. The rates r_n is rupture rate and g_n is rebinding rate as defined in Eq. 2.3.3. Here the influence of other processes(reactions) on the rate of rebinding of N_t $(t > 0)$ is assumed to be time independent and incorporated in the rate constant g_n. To simulate the system of bond rupture and rebinding the following steps have to perform at time t[13].

i) Begin with $N(t = 0) = Nt$

ii) Generate two random number r_1 and r_2 disturbed uniformly in $(0, 1)$

iii) Compute for $\alpha_o = N(t)r_n + g_n$

vi) Compute the time for next processes(reactions) by $\tau + t$; where $\tau = \frac{1}{\alpha_o} ln[\frac{1}{r_1}]$

vii) Compute a number of the bond(s) at time $\tau + t$ by: $N(\tau + t) = N(t) + 1$

for $r_2 < \frac{g_n}{\alpha_o}$ or $N(\tau + t) = N(t) - 1$ for $r_2 > \frac{g_n}{\alpha_o}$

To justify the above processes for simulation, let us consider the probability of any one process(breaking or rebinding) that takes place in the interval $[t, t + dt]$ is equal to $\alpha_o dt$, which is given as a sum of probabilities of rupture $N(t)r(n)dt$ and rebinding $g(n)dt$. Once the next time $\tau + t$ of bonds formation or break is determine then both process have the same probability otherwise frist process occurs. The decision is passed based on second random number r_2 as which reaction is (breaking or rebinding) taks place. With each execution of Gillespie algorithm we can get the evolution of a system a calculation.

Even if, any one of execution is only a probabilistic simulation, and the chances of being the same as a particular reaction is small or none[12]. To get some practical result it should be run many times in order to calculate a stochastic mean(or variance) that tells us about the behaviour of the system[13], i,e After the first(initial)transient, a number of bonds $N(t)$ fluctuations around its mean value[14].

3.2.1 Simulation procedure

Since we are modeled the ligands - receptors interaction in the coupled chemical reactions model which is the best fitted for our breaking and rebinding of the adhesion bonds. And we applied the Gillespie algorithms for coupled processes that are association and dissociation of adhesion bonds for our simulation purpose. First we have to set the relation between monte carlo time and maximum time ,i.e $t(i) \leq t_{max}$. Then we determine the rates according to the Algorithm given in the previous section.

Thus, in the present work we consider the ligands-receptors pair of N_t number of bonds which is initially placed parallel at time $t = 0$, then as time goes t, where $t \geq 0$ the system starts it's stochastic reaction. For $t \geq 0$ the numbers of bonds are changed from N_t to $N_{\tau+t}$ and let the linked bonds to be link-bond $= n(i)$. The stochastic reactions rates are function of closed bonds, open bonds, association rate constant and the external applied force. The sum of rates are given as $\alpha_o = r_n + g_n$. The random numbers r_1 and r_2 are uniformly disturbed numbers between 0 and 1, then life time of bond is determined. The life time of the bonds in this state can be expressed as $t_{life} = \frac{1}{\alpha_o} * ln(\frac{1}{r_1})$, and which reaction is occurred decision passed depending on r_2 , that is if $r_2 \leq \frac{g(link_b)}{\alpha_o}$ then the bond may be assocated given as $n(i+1) = n(i) + 1$ or dissociation given as $n(i+1) = n(i) - 1$ at any given monte carlo time step, i. These stochastic processes continuous as longe as the above conditions satisfied and up to the linked bond goes to one.

3.2.2 kinetic Monte carlo Algorithm for adhesion band

Here we use the Algorithim of section 2.3.1. The KMCM is algorithms for simulating the time evolution of a system with know rate(from all possible rate in the system) are applied.

3.2.3 Simulation procedure

Since the bond formation and breaking are probabilistic event, the probability $P(\mu, \tau/\tau_0, x)$ is basic quantity for that next reaction occur in time interval $[\tau_0 + \tau, \tau_0 + \tau + d\tau]$ and for reaction type μ under the conditions that at time τ_0 the system is in given state x. Here the μ has two values corresponding to breaking and rebinding and the rate are constant between subsequent events. The expression $a_\mu \exp(-a_\mu \tau) d\tau$ is probability for the μ in interval $[\tau, \tau + d\tau]$. By integration 1 - $\exp(-a_\mu \tau)$ which is probability for a reaction occurring until time is τ. Therefore, Eq. 2.3.11 generate random ξ that is in $[0, 1]$ and then insert it to $\tau_\mu = \frac{ln(\xi)}{a_\mu}$ that is random variable. The time for next reaction is chosen as smallest of τ_μ, i.e,$\tau = min(\tau_\mu)$. The breaking and rebinding rates are $r(n)$ = -n*exp($\frac{F}{n}$) and $g(n) = \gamma * (N_t - n)$. The sum of rates $\alpha = r(n) + g(n)$ then the life time $\tau = \frac{1}{\alpha} ln(\frac{1}{r_1})$. Based on random numbers r_1 and r_2 the next event is expected for $r_2 \leq \frac{g(n)}{\alpha}$. Hence, $n(i+1) = n(i) + 1$ or $n(i+1) = n(i) - 1$ and $t(i+1) = t(i) + \tau$. This rule generate the trajectories with correct distribution of time and type of subsequent reaction.

Chapter 4

Results and Discussion

The organization of this chapter is as follows. In the first section, we discuss the results of the Langevin dynamics simulation performed for N parallel adhesion bonds for slow variable of $1D$ using Brownian particle dynamics for given model potential energy. Here we consider the ligand-receptor pairs as Brownian particle and Brownian particle dynamics model is used which we discussed previously. In this section we will see how the position of the particle (ligand-receptor pair) can be determined from the Langevin equation (LE) using Runge Kutta fourth order method. In the next section, we see how the free energy landscape of the adhesion bonds cluster can obtain from force-displacement relation, that depict the dissociation of the adhesion bonds energy landscape. The stochastic simulation of adhesion bonds breaking and rebinding by means of the Gillespie algorithm is discussed. Then finally we will see the number of adhesion bonds n as a function of random life time realizations of the SSA.

4.1 Results from Brownian Particle Dynamics

In this section, we explain the dynamics properties and we can see this by fixing substrate position's. Thus, during our simulation we fixed the position of substrate, Brownian's particle position is shown as function of monte carlo time step. As discussed in the

chapter three and from the Brownian's particle theory that is a particle in a fluid under goes random motion (Brownian motion)in two dimension in the force field, that arises due to the interaction forces such as drag, collision and interaction potential of the particles. The parameters used for simulation purpose are: Time step increment is $dt \sim 0.08$, elastic constant of spring is $k_s = 5$, number of the particles are $N = 50$, the energy barrier is $E_b^0 = 10k_\beta T$, where $k_\beta T = 1$ and the monte carlo time steps are $i = 376$. As can see in the figure 4.1 the particles motion(diffusions)are random and the mean value is approximately equal to zero that is in agreement with theory.

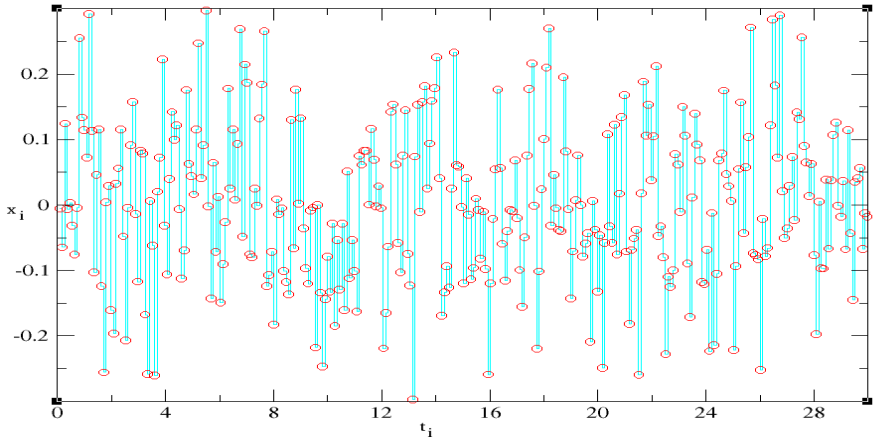

Figure 4.1: The position - monte carlo time plot of Brownian particles(ligand - receptor) of N ligand - receptor pairs interaction.

4.1.1 The energy landscape of ligand-receptor interaction

Here we present and discuss the result from our simulation. Through invesitigatoin the dependence of the particle's posistion x_i on substrate posistion X_o and the number of ligand - receptor pairs N are basic factors for the simulation. For each step of substrate's posistion there are many steps of ligands - receptors motion. As explained in chapter three the ligands-receptors interaction potential can be seen from the Langevin dynamics simulation of the model interaction potential energy. Understanding the conformational dynamics of ligands-receptors intraction requires a detailed knowledge of the structure of

their potential energy landscapes which can be seen by means of simulation only. Here the posistion of substrate is increment by 2 monte carlo unit step, for that each increment the posisition of ligands-receptors pairs are various that leads to other state of the system. The interaction range of ligands-receptors in our case is 0.02. This force-displacement relation is to obtain the free energy landscape of the ligands-receptors adhesion bonds interaction as it is clear from Eq. 3.2.2. As can be seen from the simulation result free energy landscape is useful in studying the dissociation of the ligands-receptors. The figure 4.2 shows that the peak of $F(x_o)$ is skewed some what because when force is large most of the bonds are closed, where as the posistion x_i small.

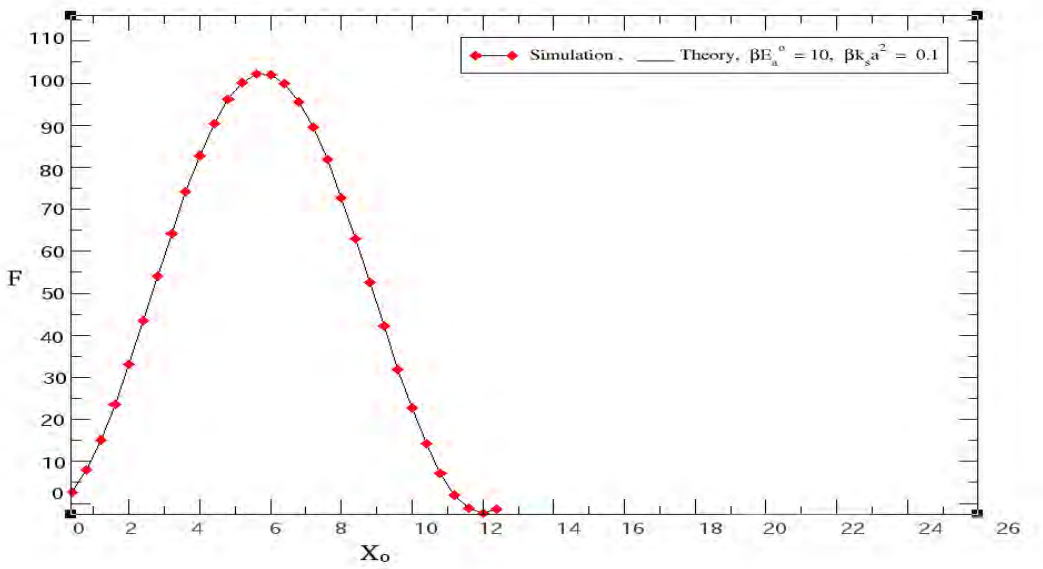

Figure 4.2: The force - posistion of substrate plot of ligand - receptor pairs interaction. The $F(X_o)$ for the $E_a^0 = 10K_\beta T$ and $k_s a^2 = 0.1k_\beta T$. The points are from LDS(N= 50) the curve is from equation.3.0.4

4.2 Dependence of adhesion bonds on number of bonds

In this section, we shall see the result from our simulation, the dependence of the adhesion bond on the external applied force F, on the number of bond, N_t, closed numbers bonds, N_b and rebinding constant, γ (as function of closed bonds, open bonds, association rate

constant and the external applied force). As clearly stated in Simulation procedure in section 3.2.2. Figure 4.3 below illustrates the breaking of a ligands-receptors pairs for different number of bonds and for N_t equal to, $N_t = 25, 50, 60, 100, 500$ and the rebinding rate constant $\gamma = 100$. As it can be seen, when the bond's number increased the lifetime of adhesion bond becomes longer we can see this by applying equal force. Thus, we can see an inverse relation between the cluster size and the external applied force. This is the representation of our stochastic model for an adhesion bonds cluster under shared force. Which is in agreement with our model where the cooperative among the bonds is assumed.

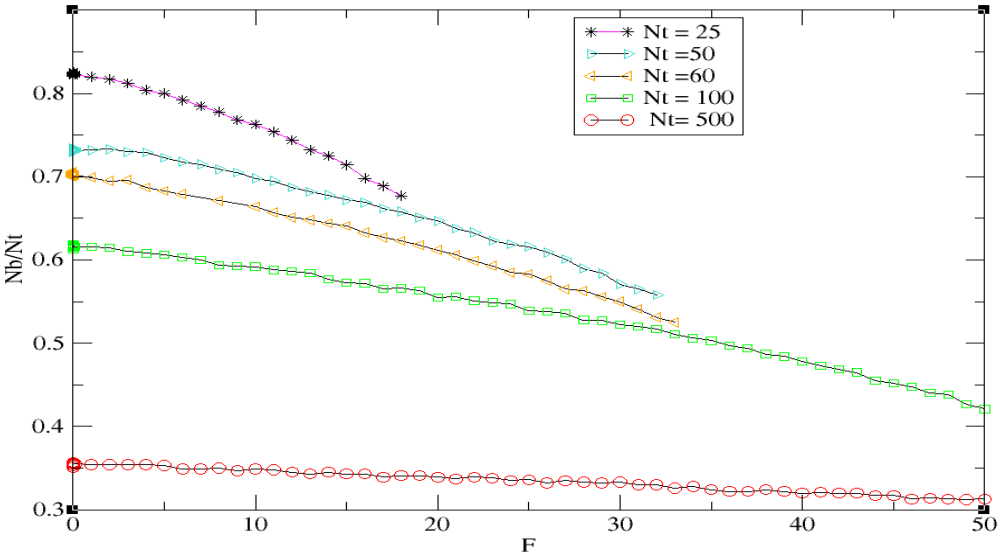

Figure 4.3: The $\frac{N_b}{N_t}$ versus external applied force which illustrates how the external applied force is shared among closed bonds. And also shows how lifetime of adhesion bond is depend on size of cluster.

4.2.1 Dependence of adhesion bonds on rebinding rate constant

Ligands-receptors rebinding rate constant influences the lifetime of the adhesion bonds. As can see from the figure 4.4 at the beginning, where the rebinding rate constant is small and the $\frac{N_b}{N_t}$ rise slowly, and then stayed approximately constant as γ increases. Figure

4.4 below illustrates how the ligands-receptors pairs interaction become nearly stable for different number of bonds, $N_t = 50, 60, 100, 200, 500$, for different rebinding rate constant γ and for external applied $F = 10^{-5}$.

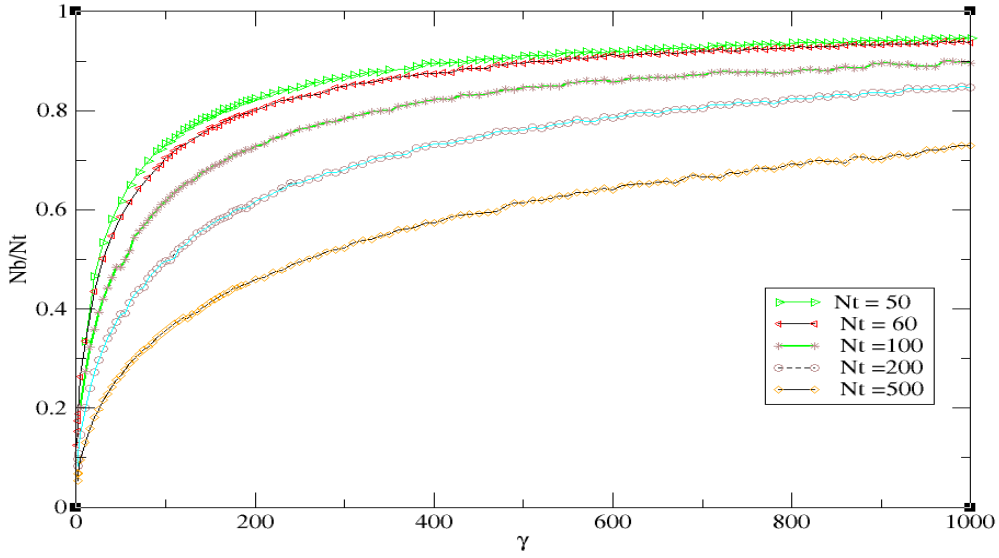

Figure 4.4: The $\frac{N_b}{N_t}$ vs γ which illustrates how the rebinding rate constant influences the existence of the bonds. And also shows how lifetime of adhesion bond is depend on size of cluster.

With every execution of Algorithm of section 3.2.1 we get the evolution of the system with it's distinict state, since any of the execution is only a probabilistic simulation, and the chances of being the same as a particular processes (reaction) is small or none[12]. So we got practical result by run many times in order to calculate a stochastic mean(or variance) that tells us about the behaviour of the system[13], i,e After the first(initial)transient, a number of bonds, $N(t)$, fluctuations around its mean value[14]. As can see easily for the cluster big in size the fluctuation become smaller and the rupture event are concentrated around the rupture of the deterministic cluster.

4.2.2 Adhesion bonds as function of random lifetime

In this section, we will see how the realizations of every interaction various with lifetime. The fraction of the bonds in the ensemble that remain intact (not changed) at time t is the survival probability of the bond.

At time $t = 0$, the number of bonds $N(t = 0) = N_t$, but for $t \geq 0$ the number of adhesion bond is equal to N_t with probability $P_n(t)$. Hence, at the beginning half of total numbers of bonds $\frac{N(t)}{2}$, are considered as linked. After the reaction started it's the average number of adhesion bonds decline fast to certain value there it fluctuate that can be seen from the figure 4.5. This is due to forward and reverse processes. There only jumps between adjacent site is allowed. Here the values of parameters taken for the processes are:the numbers of bonds $N_t = 1000$, the rebinding rate constant $\gamma = 1$, external applied force $F = 10^{-4}$, a numbers of experiment, $N_{trial} = 1000$ and the maximum time $t_{max} = 0.5$.

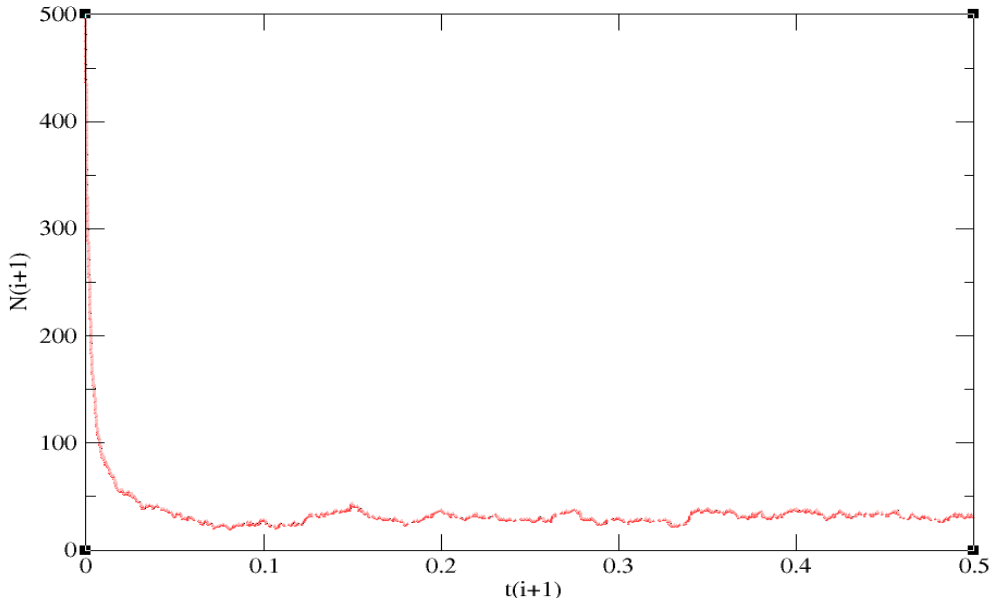

Figure 4.5: $N(i+1)$ vs $t(i+1)$ illustrates the adhesion bonds as function of random life time and the lifetime of adhesion bonds dependence on the rates.

4.3 Conclusion

In this paper we presented and tested Langevin dynamics and Gillespie Algorithm simulations for the adhesion bonds cluster. Due to the concept of stochastic nature of adhesion bonds, it has the advantage of giving real random nature in one dimensions and fitting for simulations.

We presented adhesion bonds breaking and rebinding rates in thermal equilibrium where external applied forces are present. We addressed the adhesion bonds problem by simulations. Our simulations results show that accurate estimates for the dependence of adhesion bonds on the numbers bonds, rebinding rate constant and external applied force.

We have also studied the dynamical behaviors of a adhesion bonds cluster. We have seen the free energy landscape and displacement of adhesion bonds by simulation. We saw the random nature of adhesion bonds formation, breaking and simulations results. The probabilistic process plays a major role in the bonds life time process. In the adhesion bonds process we investigated the relationship between the escape(bonds breaking) time with different parameters that influences the process. Some of these parameters are the numbers of adhesion bonds N_t, the external applied forces F, and rebinding rate constant, γ and reaction probability, $p_n(\tau)$. At presence of the energy barrier for bonds breaking and we consider the external applied force, the thermal energy and random force(the arise due to Brownian particle collision). Indeed, we observe the probabilistic reaction of adhesion bonds. Thus, we studied the unbiased adhesion bonds reaction dynamics mainly by seeing the random life time (escape time) and reaction rates.

To this end our simulations results show that accurate modelling for the adhesion bonds stochastic nature and the random escape time as a function of N_t, rebinding rate constant γ and external applied force, F.

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Declaration

This thesis is my original work, has not been presented for a degree in any other University and that all the sources of material used for the thesis have been dully acknowledged.

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This thesis has been submitted for examination with my approval as University advisor.

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