FLUCTUATION-DRIVEN TRANSPORT OF MOLECULES IN AN ASYMMETRIC MEMBRANE CHANNEL

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To my mother Akuye .

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Abstract

Aquaglyceroporin GlpF selectively conducts water and linear polyalcohol, such as glycerol across the inner membrane of Escherichia coli, with absolute exclusion of ions and charged solutes and without dissipation of the electrochemical potential across the cell membrane. There is a current interest in the study of the transport of glycerol inside the GlpF specially after the structure of the PMF of GlpF is determined by SMD simulations. Using this structure, Schulten and Kosztin [7] have numerically studied the behavior of the current density by considering various cases of external tilting force. Being interested in seeking analytical results, we model the potential of GlpF by a ratchet potential and obtain analytical expression for the current density of glycerol through GlpF for various cases of interest. In addition we compare our results with the numerical results obtained in [7] and identify regions where the analytical and numerical results agree.

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Chapter 1

Introduction

The study of interplay of noise and nonlinear dynamics presents many challenges in systems under nonequilibrium conditions. A nonequilibrium system is one in which there is a net energy flow from an external source. Many different models employing stochastic processes in physics and biology have lead to the discovery of several noise induced phenomena in systems far from equilibrium. Noise or fluctuation arises either because of the coupling of systems to an external system or from the thermal bath to which the system is in contact with. The presence of noise can alter the behavior of systems similar to what happens in a chaotic system. In contrast to the general notion that noise is undesirable and destructive, in many nonequilibrium systems it plays a constructive and stabilizing role in the dynamics. Hence noise acts as a generator of order as opposed to generator of disorder.

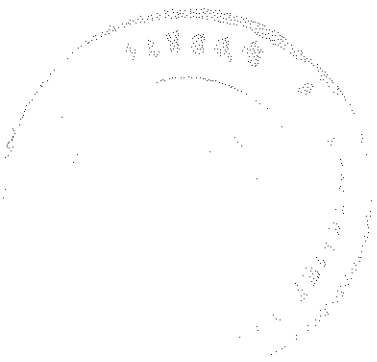
Noise-induced active transport in a fluctuating environment can arise from the so-called ratchet mechanism. Here nonequilibrium fluctuation added with spatial or temporal anisotropy conspire to generate systematic motion even in the absence of net bias.

The problem of rectification at Brownian scale was posed in 1992 by Smoluchwoski [1] and much later in a clear version by Feynman [2]. Our physical intuition, formed by everyday observation of large machines, fails when we consider the world of the small.

It is a capricious world, ruled by thermal and quantum fluctuations. For molecules, moving deterministically it is like trying to walk in a hurricane: the forces propelling a particle along the desired path are puny in comparison to the random forces exerted by the environment. Yet cells thrive. They ferry materials, they pump ions, they build proteins, they move from here to there. They make order out of anarchy. Over the past several years researchers have finally begun to understand how this happens in a noisy environment. The basic insight, loosely described as the Brownian ratchet principle, is that random noise can be put to good use. The trick is to rectify the noise, to filter out the randomness you do not want so that you are left with what you do want. This principle resembles the phenomenon known as stochastic synchronization, where increasing the noise in communication channels can actually make it easier to transmit a signal.

In recent years, an increasing number of biological processes which were thought to have resulted from directed movement have been discovered to employ biased Brownian movement. A striking example is the movement of myosin motor proteins along actin filaments to generate the force of muscle contraction [3]. The popular model for actomyosin force generation is that the myosin heads move along the actin in a deterministic, controlled manner. However, this indicates that the myosin heads actually use energy stored in ATP to hop along the actin ranging from 5.5 nm to 27.5 nm [4]. Occasionally, the myosin can even move backwards. The net direction of movement is forward but this research puts into question the traditional model of controlled, incremental forward movement of this biomolecular machine [5]. Another example of Brownian motor involves the processive movement of a single kinesin molecule along a microtubule [3]. In order to facilitate the transport of vesicles in the cell, kinesin molecules bind to these vesicles and then move along microtuble to deliver the vesicle to its proper location. The popular explanation for this movement has always been that the kinesin has a deterministic, controlled walking motion by its

two heads along the microtubule. However, researchers have shown that the kinesin can walk along the microtubule even if one of the heads is disabled. This observation does not fit in the popular model which requires two heads to walk together along the fibers [6]. However, it fits nicely with the Brownian model. In this example the tubulin molecules, which are the individual monomers that make up the microtubules, pose an energy barrier to the movement of kinesin. Without outside energy, kinesin is stuck on the tubulin molecule it finds itself on. The hydrolysis of an ATP molecule lowers this energy barrier so that the kinesin can rattle back and forth along the microtubule, but in a preferentially forward manner. When the hydrolysis products of ATP are released, the kinesin locks in whatever gains it made until the next ATP is hydrolyzed. In this way, the processive movement of kinesin along the microtubule results from random, yet directed motion. The motor molecules are used for the transportation of organelles (cargo, chemicals) for intracellular transport and muscle contraction or to power muscles. The energy source of these molecules comes from the hydrolysis of ATP. In ATP energy is stored in the phosphate bonds, this energy is released when the bond is hydrolyzed and ADP is produced. The motor proteins use this energy to bring about unidirectional motion along the biopolymers. Hence chemical energy is converted to mechanical energy. The fluctuation in the potential experienced by the motors are believed to arise from the binding and dissociation of ATP, and the anisotropic periodic potential as representing the electrostatic potential along the long structural filament. Moreover, it is also clear that the motion of these motors can be described as an overdamped motion of Brownian particles. At any time the velocity of the particle is proportional to the force on the particle. These particles experience random kicks from the surrounding medium and the average thermal energy of a particle is k_BT. This energy k_BT is comparable to the other involved energy scales in the problem, such as the barrier heights. Hence, Brownian motion plays an essential role in the action of motors. Thus, protein motors operate in



a Brownian regime where inertia is negligible and thermal fluctuation are important. Cell metabolism requires controlled molecular transport across the cell membrane, a function that is fulfilled by a wide variety of transmembrane proteins, acting as passive and active transporters [7].

Membrane proteins are typically difficult to characterize structurally because the requirement for maintaining a membrane environment hinders purification and crystallization. The recent progress in the structural and functional characterization of several membranes of the K^+ channel [8] provide us with a unique opportunity to explain the function of these biomolecular systems. Although the experimentally determined three-dimensional structure of membrane channels yields a wealth of information, membrane transport processes are intrinsically dynamical and theoretical considerations are necessary for understanding the underlying mechanisms of selection and conduction.

Escherichia coli, like all bacteria, has evolved mechanisms that allow it to survive in the most nutrient-poor conditions. One such mechanism is a very efficient glycerol uptake systems that allows rapid growth in glycerol solution at low concentrations. Glycerol molecules entering the cell pass through two types of passive channel proteins; first the porins, located in the outer membrane, then the inner-membrane aquaglyceroporins, including the glycerol facilitator GlpF. Upon entering the cytoplasm, glycerol molecules are phosphorylated by glycerol kinase and are unable to diffuse out of the cell [7]. GlpF belongs to the aquaporin family, a family of channels for water and small hydrophilic solutes which is found in most organisms. In addition to water, GlpF conducts linear polyalcohols with a high degree of stereoselectivity [7]. The recent discovery of the structure of GlpF at a resolution of $2.2\mathring{A}$ [9] has revealed important features of the transport mechanism. The channel walls match the hydrophilic and hydrophobic sides of glycerol, so that glycerol can be dehydrated

without an energy penalty. There have been early attempts to reconstruct potential mean force (PMF) from steered molecular dynamics (SMD) simulations [10]; the suggested methods, however, either neglected the nonequilibrium character of the simulations or required knowledge of the friction coefficient which is generally unknown. The recently determined three-dimensional structure of GlpF at atomic resolution [7] has provided much insight into the underlying microscopic mechanism of molecular transport and selectivity through GlpF [11]. In particular, molecular dynamics (MD) simulation studies [12] established that water and glycerol diffusion through GlpF is a single file, and for biologically relevant periplasmic glycerol concentration correlation effect between consecutive glycerol molecules are negligible due to their large spatial separation. The inner lining of GlpF consists of hydrophobic face complimented by hydrogen-bonding groups on the opposite side matching closely the amphiphilic structure of glycerol and other linear sugar molecules. The narrowest region of the channel is located close to the periplasmic mouth of the channel, and is proposed to function as its selectivity filter [11]. The permeation of glycerol through GlpF is controlled largely by energetics. The molecule needs to be attracted to the channel and as it transits the channel it must be subjected to interactions that select it, rejecting other components that may also fit in the channel. One expects the energetics to be cast into an energy profile which exhibits attractive well at the ends of the channel and barriers in the selectivity regions of the channel, the barrier being surmountable by glycerol and related molecules, but too high for other molecules.

The conduction of glycerol through GlpF has been studied numerically by using the potential of GlpF from molecular dynamics simulation by Schulten and Koszstin [7]. In their work the flux is calculated at steady state in different cases. They proposed and showed that under realistic physiological condition, the asymmetry of GlpF furnishes active transport through the ratchet mechanism. It was also

found that, as a result of channel asymmetry glycerol uptake, driven by concentration gradient, is enhanced significantly in the presence of nonequilibrium fluctuations. They also studied the effect of attractive vestibule in the periplasmic side, by inverting the orientation of the channel. In this case they studied the fluxes through the normal and inverted channel as a function of periplasmic glycerol concentration and an external load. They also studied the effect of nonequilibrium fluctuation on glycerol uptake, by taking a periodically switching load. To do this, they studied the nature of the fluxes as a function of the load, by taking different conditions.

Some other models that simplify the PMF of GlpF, have been employed: the six steps model and the two steps model. The six steps model is inspired by the results of SMD simulations, which indicate that glycerol makes a series of discrete steps as it passes through the channel. In the two steps model the details of the PMF are ignored except for the barrier and well. The two steps model is useful to get analytical expression for the net flux.

We approximate the PMF of GlpF, which was obtained by SMD simulations, by ratchet like potential to study the conduction of glycerol analytically. In this model all the details of the PMF are neglected except for the well and a barrier. Using the approximate model we evaluate glycerol conduction at steady state and compare the result with that obtained numerically [7].

The rest of this thesis is organized as follows. In chapter two, we calculate the steady state flux through GlpF using our model potential . At steady state we assume different periplasmic and cytoplasmic concentrations. Here due to the concentration gradient we expect both inward and outward fluxes. To study the effect of an external non equilibrium fluctuations on glycerol conduction, we take different cases of external load: zero load, constant load, and time dependent periodic load.

In the presence of an external periodic load, we compare the net flux, inward flux, and outward flux with the flux with zero load and no cytoplasmic concentrations. We

also study the populations at the ends of the channel in the case of no net flux, and their dependencies on an external load. We study the effect of the potential well on the periplasmic side on glycerol uptake. To do this we invert the orientation of the channel, i.e., the well facing the cytoplasmic side. To see this effect, we compare the fluxes through both channels as a function of periplasmic glycerol concentrations. We also study their ratio as a function of an external load.

Using our model, we also study the effects an external load and asymmetric parameters of the potential have on the fluxes and populations at the ends of the channel. In all cases we show that our analytical work agrees with numerically obtained results for a limited range of an external load and asymmetric parameters of the potential.

In chapter three we discuss the results we obtained analytically using the model potential of GlpF proposed and compare with those obtained numerically using the PMF of GlpF by Schulten and Kosztin [7]. In chapter four we give a brief summary and conclusion of our work.

Chapter 2

Transport of glycerol inside GlpF

In this chapter, we model the potential of Escherichia glycerol uptake facilitator(GlpF) by a ratchet potential and study the conduction of glycerol molecules inside this channel. Glycerol molecules are considered as Brownian particles moving in the model potential (with and without external load). We consider the medium through which the glycerol molecules move to be of high friction. We study the dependence of the flux on the external load and on the concentration gradient at the ends of the channel by taking different cases. In the first section of this chapter we derive an expression for the steady state flux of an arbitrary potential with the presence of an external load and concentration gradient. In the second section we take our model and find an exact expression for the steady state flux. In the third section we consider a particular case where the load is zero. We will also explore how this flux depends on the concentration gradient that describes the model. In the fourth section we will consider our model in the presence of a constant external load, and explore how it affects the flux. In the fifth section we will simplify our model by taking a load that switches periodically between $+F$ and $-F$ and study the nature of the fluxes. The nature of the populations at the ends of the channel will be explored in the case of no net flux. The nature of the inward, outward and average fluxes with respect to the flux with zero load and no cytoplasmic concentration, as a function of parameters

describing the model will be studied. We will also find the range of the external load, with some choice of the parameters, for which the condition is favorable to the survival of the cell.

2.1 Steady state probability current for arbitrary potential

We consider the motion of an overdamped Brownian particle, along the axis of a channel of potential $U(x)$. The motion of Brownian particle, in the presence of an external force $F(t)$ in the strong friction limit is described by the Langevin equation [13]

$$\gamma\dot{x} = f(x) + \xi(t) + F(t), \tag{2.1}$$

where γ is the friction coefficient, $f(x) = -U'(x)$ is the deterministic force derived from the potential, and $\xi(t)$ is the random thermal kick of the medium such that, $< \xi(t) >= 0$ and $< \xi(t)\xi(t') >= 2k_BT\gamma\delta(t-t')$. $\delta(t-t')$ is the Dirac-delta function, and D is the diffusion coefficient of glycerol molecules inside GlpF. According to the fluctuation dissipation theorem, D and γ are related through the Einstein relation, $D = k_BT/\gamma$, where T is the temprature of the medium. We assume that F is an external force to be taken as either a constant or a periodically switching load depending on the problem.

The Fokker Planck equation corresponding to the above Langevin equation is given by

$$\frac{\partial}{\partial t}P(x,t) = \frac{1}{\gamma}\frac{\partial}{\partial x}[V'(x)P(x,t) + k_BT\frac{\partial}{\partial x}P(x,t)] = -\frac{\partial}{\partial x}J(x,t), \tag{2.2}$$

where, $J(x,t)$ is given by

$$J(x,t) = -\frac{1}{\gamma}k_BT\frac{\partial}{\partial x}P(x,t) - \frac{1}{\gamma}V'(x)P(x,t), \tag{2.3}$$

where $V(x) = U(x) - Fx$ is the effective potential of the system. The steady state solution, $P^s(x)$ of the Smoluchowski equation implies a constant current, J, given by,

$$J = [f(x) + F]P^s(x) - k_BT\frac{d}{dx}P^s(x). \tag{2.4}$$

Here, we assume that the probability is not conserved due to the concentration gradient,

$$P^s(0) \neq P^s(L), \tag{2.5}$$

and the effective potential $V(x)$ does not have the same value at the ends of the channel due to the load,

$$V(0) \neq V(L). \tag{2.6}$$

The stationary probability distribution can be given as

$$P^s(x) = B(x)e^{-\phi(x)}, \tag{2.7}$$

where $B(x)$ is consistently to be determined and while

,

$$\phi(x) = \frac{1}{k_BT}\int_0^x (f(x') + F)dx'. \tag{2.8}$$

After multiplying equation (2.4) by $e^{\phi(x)}$ we can write it as

$$\frac{d}{dx}(e^{\phi(x)}P^s(x)) = -\frac{1}{k_BT}Je^{-\phi(x)}. \tag{2.9}$$

Integrating equation (2.9) from 0 to x , we get

$$P^s(x) = e^{-\phi(x)}\{P^s(0)e^{\phi(0)} - \frac{1}{k_BT}J\int_0^x e^{\phi(x')}dx'\}. \tag{2.10}$$

Hence, B(x) of equation (2.7) is the factor appearing in the big bracket of equation (2.10).

Taking $x = L$ in equation (2. 10) and solving for J, we obtain

$$J = k_B T \frac{P^s(0)e^{\phi(0)} - P^s(L)e^{\phi(L)}}{\int_0^L e^{\phi(x)}dx}, \tag{2.11}$$

with

$$e^{\phi(0)} = 1, \tag{2.12}$$

and

$$e^{\phi(L)} = e^{-FL}. \tag{2.13}$$

Equation (2.11) can be written as

$$J(F|P^s(0), P^s(L)) = P^s(0)A_0(F) - P^s(L)A_L(F), \tag{2.14}$$

where,

$$A_0(F) = \frac{1}{I_1 + I_2 + I_3}, \tag{2.15}$$

with I_1 , I_2 and I_3, respectively, given by

$$I_1 = \frac{L_1(1 - e^{-(Q+L_1F)})}{Q + L_1F}, \tag{2.16}$$

$$I_2 = \frac{(L_2 - L_1)(e^{-(Q+FL_1)} - e^{Q-FL_2})}{-2Q + F(L_2 - L_1)}, \tag{2.17}$$

and

$$I_3 = \frac{(L - L_2)(e^{Q-FL_2} - e^{-FL})}{Q + F(L - L_2)}. \tag{2.18}$$

In addition $A_L(F)$ is given by

$$A_L(F) = \frac{e^{-FL}}{I_1 + I_2 + I_3}. \tag{2.19}$$

2.2 Steady state flux of glycerol molecules inside GlpF

The section through the glycerol conduction pathway in GlpF and the corresponding asymmetric PMF obtained by MD simulations is shown in Fig. 2.1, with a prominent potential well at the external (cytoplasmic) side and a constriction region with several pronounced potential barriers towards the internal (cytoplasmic) side of the channel.

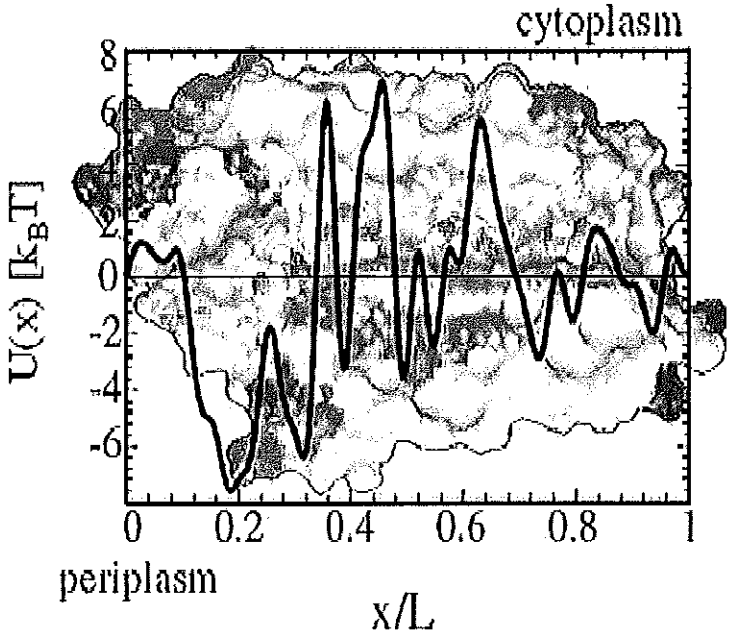


Figure 2.1: Section through the glycerol conduction pathway in GlpF and the corresponding asymmetric PMF obtained by SMD simulations as reported in [7] .

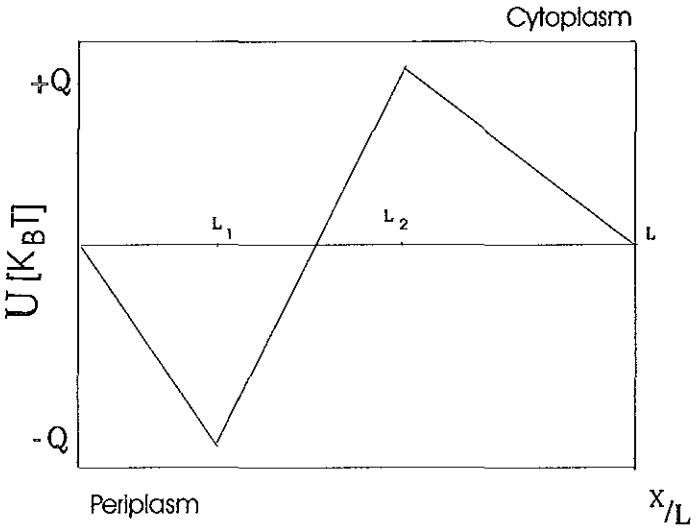


Figure 2.2: Plot of the model potential versus position.

In this section, we study the conduction of glycerol molecules through GlpF analytically, by taking a simplified model potential (ratchet potential) instead of the PMF of GlpF shown in Fig. 2.1. The model potential of GlpF, $U(x)$ is given by

$$U(x) = \begin{cases} \frac{-Qx}{L_1} & \text{if } 0 \leq x \leq L_1, \\ \frac{2Q(x-L_1)}{L_2-L_1} - Q & \text{if } L_1 \leq x \leq L_2, \\ \frac{-Q(x-L_2)}{L-L_2} + Q & \text{if } L_2 \leq x \leq L. \end{cases} \tag{2.20}$$

At this point we introduce dimensionless units that will be employed throughout this paper, unless otherwise stated. All other units can be expressed in terms of the following, length of GlpF $L \approx 4.8nm$, diffusion time $\tau_D = L^2/D \approx 10^{-7}s$, and thermal energy $\varepsilon = k_B T \approx 4.28 \times 10^{-21}J$, here k_B is the Boltzmann constant, $T = 310K$ is the physiological temperature, and $D \approx 2.2 \times 10^{-10}m^2/s$ is the diffusion coefficient of glycerol inside GlpF [7]. Thus, the force unit is $F = \frac{\gamma D}{L} = k_B T/L \approx 0.9pN$. The probability $P(x)$ is related to the local concentration $C(x)$ by

$$P(x) = C(x)S(x), \tag{2.21}$$

where $S(x)$ is the area of the channel cross section. From the crystal structure of GlpF, it was found that the opening area at both ends is $S(0) = S(L) \approx 100\mathring{A}^2$. In the new units, the steady state flux of equation (2.11) can be written as

$$J = \frac{P^s(0)e^{\phi(0)} - P^s(L)e^{\phi(L)}}{\int_0^L e^{\phi(x)}dx}, \tag{2.22}$$

with

$$e^{\phi(0)} = 1, \tag{2.23}$$

and

$$e^{\phi(L)} = e^{-FL}, \tag{2.24}$$

equation (2.22) can be written as

$$J(F|P^s(0), P^s(L)) = P^s(0)A_0(F) - P^s(L)A_L(F), \tag{2.25}$$

where $A_0(F)$ is given by

$$A_0(F) = \frac{1}{I_1 + I_2 + I_3}, \tag{2.26}$$

where I_1 , I_2 and I_3 are given, respectively, as

$$I_1 = \frac{L_1(1 - e^{-(Q+L_1F)})}{Q + L_1F}, \tag{2.27}$$

$$I_2 = \frac{(L_2 - L_1)(e^{-(Q+FL_1)} - e^{Q-FL_2})}{-2Q + F(L_2 - L_1)}, \tag{2.28}$$

$$I_3 = \frac{(L - L_2)(e^{Q-FL_2} - e^{-FL})}{Q + F(L - L_2)}. \tag{2.29}$$

On the other hand $A_L(F)$ is given by

$$A_L(F) = \frac{e^{-FL}}{I_1 + I_2 + I_3}. \tag{2.30}$$

2.3 Zero load

In this section, we consider our model potential and study glycerol uptake in the absence of load. $U(x)$ is completely determined by the parameters: Q, L_1 and L_2. The barrier height is located at $x = L_2$ and the minima at $x = L_1$. In this case (zero load), the potential $U(x)$ vanishes on both ends of the channel.

$$U(0) = U(L) = 0. \tag{2.31}$$

In this case the flux is given by

$$J(P^s(0), P^s(L)) = A_0(0)(P^s(0) - P^s(L)) = A_0(0)S(0)(P^s(0) - P^s(L)), \tag{2.32}$$

Where A_0 is given by

$$A_0(0) = \frac{1}{R_1 + R_2 + R_3}, \tag{2.33}$$

Where,

$$R_1 = \frac{1 - e^{-Q}}{Q}, \tag{2.34}$$

$$R_2 = \frac{(L_2 - L_1)(e^{-Q} - e^{Q})}{-2Q}, \tag{2.35}$$

and

$$R_3 = \frac{(L - L_2)(e^{Q} - 1)}{Q}. \tag{2.36}$$

One has $A_0(0) = A_L(0)$, and the flux is proportional to the concentration gradient, but insensitive to the asymmetry of the potential.

2.4 Non-zero constant Load

In this section, a particular case where there is a static external force is considered. Due to the load there established a potential gradient at the ends of the channel.

$$V(0) \neq V(L). \tag{2.37}$$

Due to this potential gradient, a dependence of the flux on the asymmetry of the potential is observed. In this case the flux through the channel depends on both the load and concentration gradient. The net flux in the presence a constant load is given by

$$J(F|P^s(0), P^s(L)) = J^{>} - J^{<}, \tag{2.38}$$

where $J^{>}$ and $J^{<}$ are the inward and outward fluxes respectively, and

$$J^{>} = A_0(F)P^s(0), \tag{2.39}$$

and

$$J^{<} = A_L(F)P^s(L), \tag{2.40}$$

where $A_0(F)$ and $A_L(F)$ are given in equation (2.26) and equation (2.30), respectively. We define, J_0 as the flux generated in the absence of both load and cytoplasmic concentration by

$$J_0 = J(0|P^s(0), 0) = A_0(0)P^s(0), \tag{2.41}$$

where $A_0(0)$ is given by equation (2.33).

2.5 Periodically switching load

In this section, we consider a load that switches periodically between $+F$ and $-F$, and study its effect on the flux and populations at the ends of the channel. Although the time average of the force is zero, it induces finite current through the channel. The mean flux through the channel is expressed as [14]

$$\bar{J} = \frac{1}{2}[J(F|P^s(0), P^s(L)) + J(-F|P^s(0), P^s(L))], \tag{2.42}$$

where, $J(F|P^s(0), P^s(L))$ is given as in equation (2.38).

equation (2.42) can be written as

$$\bar{J} = \frac{1}{2}[P^s(0)(A_0(F) + A_0(-F)) - P^s(L)(A_L(F) + A_L(-F))]. \tag{2.43}$$

Further equation (2.43) can be written as

$$\bar{J} = \frac{1}{2}[J^{>} - J^{<}], \tag{2.44}$$

where $J^{>}$ and $J^{<}$ are the inward and outward fluxes, respectively, and given by

$$J^{>} = P^s(0)(A_0(F) + A_0(-F)), \tag{2.45}$$

and

$$J^{<} = -P^s(L)(A_L(F) + A_L(-F)), \tag{2.46}$$

where $A_0(F)$ and $A_L(F)$ are given by equation (2.26) and equation (2.30) respectively. And,

$$A_0(-F) = \frac{1}{G_1 + G_2 + G_3}, \tag{2.47}$$

where G_1 , G_2 and G_3 are given respectively as

$$G_1 = \frac{L_1(1 - e^{-(Q - L_1 F)})}{Q - L_1 F}, \tag{2.48}$$

$$G_2 = \frac{(L_2 - L_1)(e^{-(Q-FL_1)} - e^{Q+FL_2})}{-2Q - F(L_2 - L_1)}, \tag{2.49}$$

$$G_3 = \frac{(L - L_2)(e^{Q+FL_2} - e^{FL})}{Q - F(L - L_2)}. \tag{2.50}$$

On the other hand $A_L(-F)$ is given by

$$A_L(-F) = \frac{e^{FL}}{G_1 + G_2 + G_3}. \tag{2.51}$$

Then J_0 is given by

$$J_0 = \bar{J}(0|P^s(0), 0) = A_0(0)P^s(0), \tag{2.52}$$

where $A_0(0)$ is given by equation (2.34).

Chapter 3

Results and discussion

3.1 Channel with inverted orientation

GlpF is a passive channel, for which, under equilibrium conditions, the inward and outward transport rates are the same. However, the presence of such significant structure domain on the periplasm side, and not inside the cell, may be indicative of some functional implications. At physiological condition, the channel facilitates almost exclusively inward transport of glycerol, because glycerol becomes phosphorylated immediately after entering the cytoplasmic region. Trapping glycerol from the periplasm is likely to be the most important, and probably the rate limiting, step of the transport, especially under low concentration conditions: the channel must snatch glycerol while it is nearby. Identical free energies can be assumed for a solvated glycerol molecule in the cytoplasmic and periplasmic aqueous media. Nevertheless, the potential displays a clear asymmetry in the two entrance vestibules of the channel. At the periplasmic vestibule, we note a deep well, where as the corresponding site at the cytoplasmic vestibule exhibits no minimum. This asymmetry seems related to the structure of GlpF. The periplasmic vestibule is bigger and more protruding from the membrane than the cytoplasmic one. Our analysis above stresses the importance of the attractive vestibule for the glycerol conduction. But why is the vestibule on

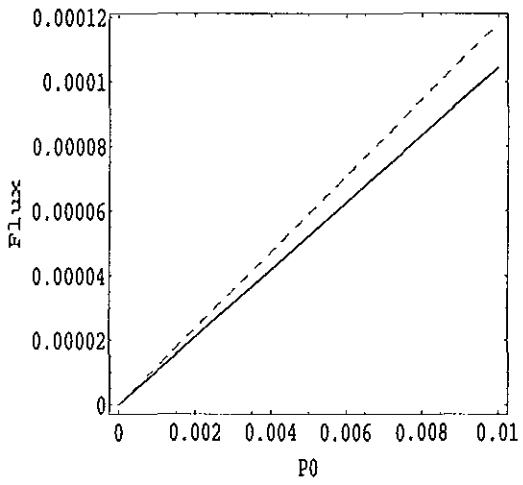


Figure 3.1: Plots of the flux through the normal channel (solid line) and the flux through the reversed channel (dashed line) versus periplasmic glycerol concentration, for $L_1 = 0.3$, $L_2 = 0.4$, $Q = 8$, $F = 2$, and $L = 1$.

the periplasmic side, and not on the cytoplasmic side? To answer this question, we consider a channel with an inverted potential so that the attractive well is facing the cytoplasmic side. In other words regarding the potential the fluxes of the normal and inverted orientation correspond to glycerol influx and out flux. The fluxes through both channels are plotted as a function periplasmic glycerol concentration. For very small concentrations the fluxes in both directions are about the same and as we increase the concentration, the flux in the reversed channel becomes larger than the flux through the normal channel Fig. 3.1.

These results can be understood in the context of simple diffusion argument. For low enough concentration, interactions between glycerol molecules can be neglected. If we define the direction of the net flux to be from periplasm to cytoplasm, the net flux is given by subtracting an outflux proportional to the cytoplasmic concentration

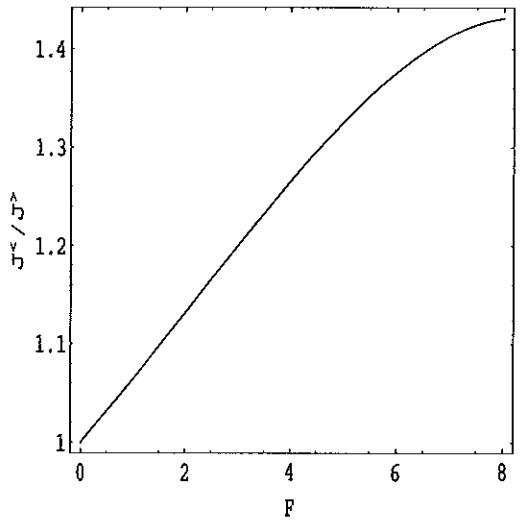


Figure 3.2: Plot of the ratio of the flux through the inverted channel to the flux through the normal channel, for $L_1 = 0.3$, $L_2 = 0.4$, $Q = 8$, and $L = 1$.

from an influx proportional to the periplasmic concentration. In static equilibrium, equal concentration of glycerol are found on both sides and there is no net flux, so the two coefficients of proportionality must be equal. Accordingly the net flux is proportional to the concentration difference, $C(L) - C(0)$, with a positive coefficient of proportionality. In a living cell, the channel conducts low concentrations of periplasmic glycerol in to the cytoplasm, which is nearly devoid of glycerol. Because the flux depends only on the concentration difference across the channel, maintaining glycerol concentration on both sides and reversing the channel will not change the flux. Therefore, there seems to be no reason to prefer one direction over the other. At higher concentration, however, interactions become more important, and conduction is no longer linear. Since glycerol molecules held in the vestibule prevent others from being conducted, the optimal direction can be estimated by putting the vestibule where it will be most rarely occupied; as close to the cytoplasm as possible. As a

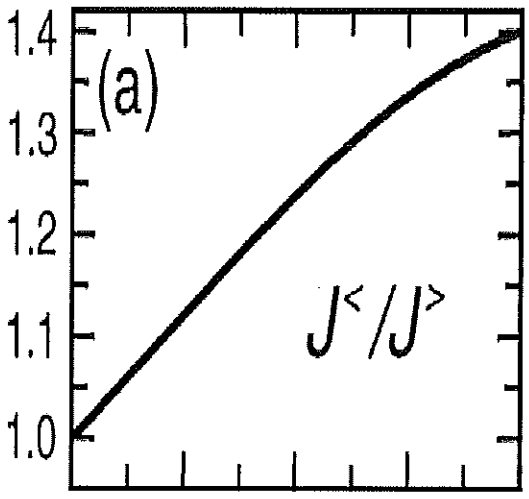


Figure 3.3: Plot of the ratio of the flux through the inverted channel to the flux through the normal channel versus load, obtained numerically using the PMF of GlpF as reported in [7] .

result, the reversed GlpF has an increased conduction rate due to reduced clogging in the vestibule. One explanation for the channel orientation of GlpF is that if the attractive vestibule would face the cytoplasm, it would be clogged by a variety of solutes inside the cell.

The ratio of the flux through the inverted channel to the flux through the normal channel is plotted as a function of load. It is found that, for $L_1 = 0.3$, $L_2 = 0.4$ it increases monotonically with the load Fig. 3.2. This result is consistent with that obtained numerical using the PMF of GlpF by Schulten and Kosztin [7] Fig. 3.3.

To see the effect of the asymmetric parameters L_1 and L_2 of our model potential on the fluxes through the normal and reversed channels, the ratio $\frac{J^<}{J^>}$ is plotted as a function of the load for $L_1 = 0.3$ and $L_2 = 0.432$, for lower values of the load the ratio decreases with the load, but for higher values of the load it increases with the

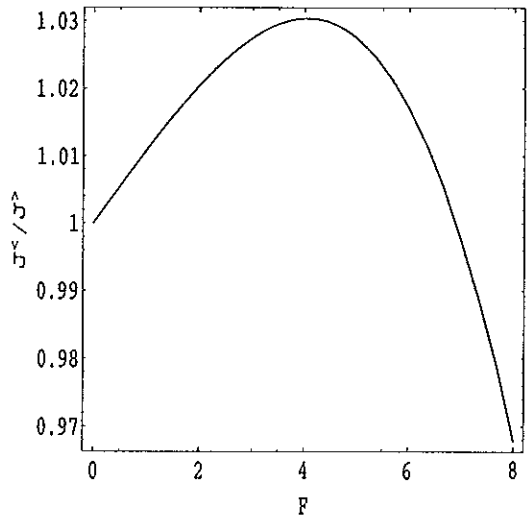


Figure 3.4: Plot of the ratio of the flux through the inverted channel to the flux through the normal channel, for $L_1 = 0.3$, $L_2 = 0.432$, $Q = 8$, and $L = 1$.

load Fig. 3.4.

3.2 Channel with normal orientation

The conduction of glycerol through the PMF of GlpF is studied numerically at physiological temperature in [7], for different cases of an external load. The ratios $\frac{J^{>}}{J_0}$ with $P(1) = 0$ and $\frac{J^{<}}{J_0}$ with $P(0) = 0$ have been studied as a function of an external load and found that both increase monotonically with the load Fig.3.5 .

For $P(1) = P(0)$ they found that $\bar{J} < 0$, and the ratio $\frac{\bar{J}}{J_0}$ decreases monotonically with the load, showing that for the same force level and concentration gradient, GlpF driven by fluctuation can transport glycerol in to the cell and protects the cell from large doses of glycerol Fig. 3.6 . The ratio of the populations at the ends of the channel is studied numerically with no net flux. The ratio decreases monotonically with the load as reported in [7] (see Fig.3.7).

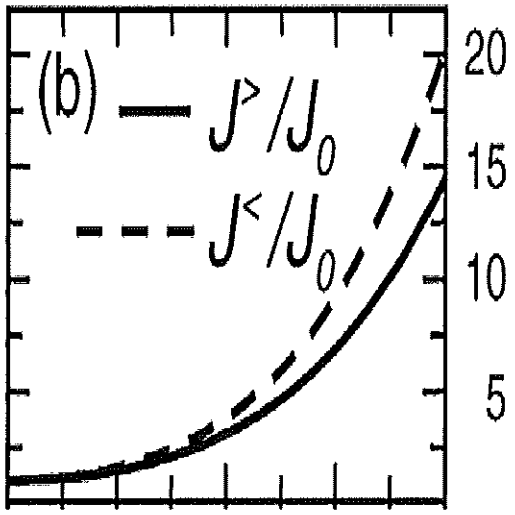


Figure 3.5: Transport induced by a square-wave force in GlpF . Shown are the inward ($P(1) = 0$), outward ($P(0) = 0$) versus load as reported in [7].

In this paper, we studied the conduction of glycerol through GlpF analytically using our model potential (ratchet potential) at physiological temperature. The model we used simplifies the actual potential of the channel that is constructed using SMD simulations. In this paper we assumed the concentrations at the ends of the channel to be different. We have included an external load and studied its effect by taking different cases. Both the concentration gradient and the external load play a significant role on glycerol uptake. In the absence of an external load, we have calculated the net flux and shown that, the flux vanishes in the absence of concentration gradient. In this case we observe that the asymmetry of the potential has no effect on the net flux. So, we conclude that non equilibrium fluctuations are necessary to induce directed transport across membrane channels.

For a periodically switching load, by taking different cases, the ratios $\frac{\bar{J}}{J_0}$, $\frac{J^{>}}{J_0}$ and $\frac{J^{<}}{J_0}$ have been studied as a function of the load.

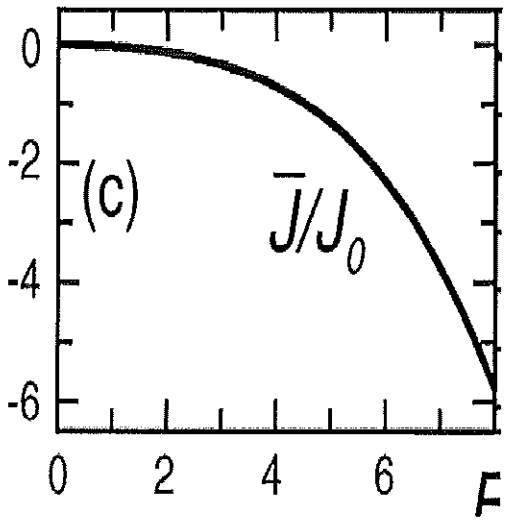


Figure 3.6: Plot of the ratio of the average flux to the flux with no load and cytoplasmic concentration versus load as reported in [7].

For equal cytoplasmic and periplasmic concentrations, $L_1 = 0.3$ and $L_2 = 0.4$ $\frac{\bar{J}}{J_0}$ is plotted as a function of the load and found that it decreases monotonically with the load Fig. 3.8.

This shows that for the same force level and concentration gradient, GlpF driven by fluctuations can transport glycerol in to the cell as well as protect the cell against poisoning through large doses of glycerol. This result Fig. 3.8 is consistent with numerically obtained result Fig. 3.6 by Schulten and Koszstin [7].

It was observed that for $L_1 = 0.3$, $L_2 = 0.432$ the effect is reversed, for small values of the load, the outward flux is bigger than the inward, but for large values of the load a reversed effect is observed, which might be an adverse effect to the cell Fig. 3.5.

The ratio $\frac{J^{>}}{J_0}$ with $P(1) = 0$ and $\frac{J^{<}}{J_0}$ with $P(0) = 0$ are plotted as a function of an external load, both increase monotonically with the load Fig. 3.10 . These results are

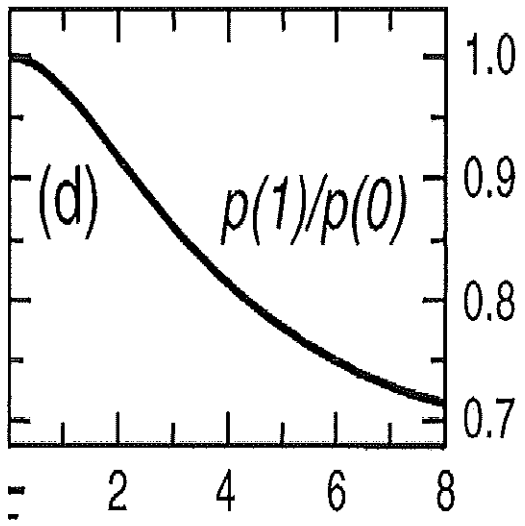


Figure 3.7: Plot of the ratio $P(1)/P(0)$ versus load with no net flux as reported in [7].

consistent with the numerical results obtained shown in Fig. 3.5. The increments of $\frac{J^{>}}{J_0}$ and $\frac{J^{<}}{J_0}$ are larger in our model than in the PMF of GlpF. This difference is due to the fact that, in our model the details of the potential are ignored except for the well and barrier which reduce clogging effect of the potential.

The increment in $\frac{J^{<}}{J_0}$ is bigger than the increment in $\frac{J^{>}}{J_0}$ which is in agreement to the fact that the outward flux should exceed the inward flux.

Due to the concentration gradient established at the ends of the channel, the probabilities of finding glycerol molecules at both ends are different. For the normal functioning of the cell, the cytoplasmic concentration should be less than the periplasmic concentration, since excess cytoplasmic glycerol concentration is harmful to the cell. For $L_1 = 0.3$, $L_2 = 0.432$ the ratio first decreases with the load and then increases for higher values of the load.

To compare the populations at both ends of the channel the ratio $\frac{p(1)}{p(0)}$ is plotted as a function of the load in the absence of net flux. For $L_1 = 0.3$, $L_2 = 0.4$ the ratio

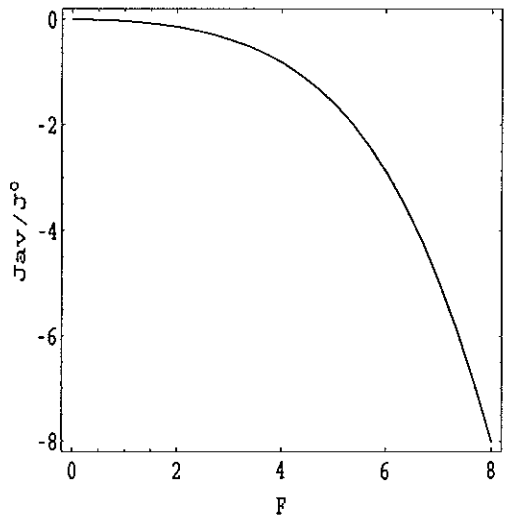


Figure 3.8: Plot of the ratio of the average flux to the flux with no load and cytoplasmic concentration versus load, for $L_1 = 0.3$, $L_2 = 0.4$, $Q = 8$, and $L = 1$.

decreases monotonically with the load Fig. 3.13, which is in a good agreement with that obtained numerically by Schulten and Kosztin [7] Fig. 3.7.

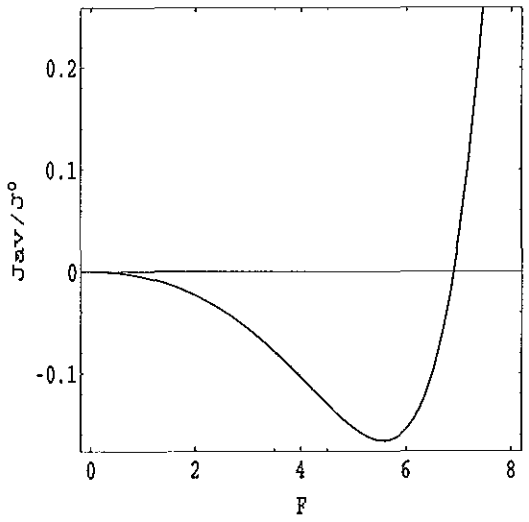


Figure 3.9: Plot of the ratio of the average flux to the flux with no load and cytoplasmic concentration versus load, for $L_1 = 0.3$, $L_2 = 0.432$, $Q = 8$, and $L = 1$.

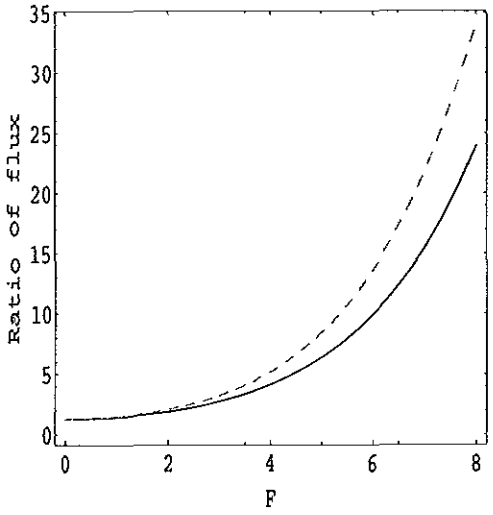


Figure 3.10: Plot of the ratio of the inward flux(dashed line) and outward flux(solid line) to the flux with no load and cytoplasmic concentration versus load, for $L_1 = 0.3$, $L_2 = 0.4$, $Q = 8$, and $L = 1$.

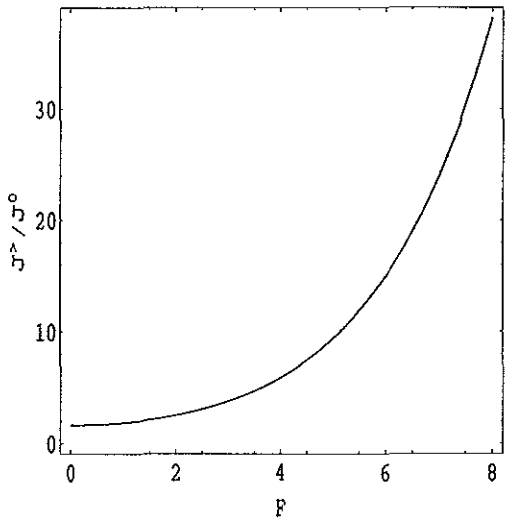


Figure 3.11: Plot of the ratio of the inward flux to the flux with no load and cytoplasmic concentration versus load, for $L_1 = 0.3$, $L_2 = 0.432$, $Q = 8$, and $L = 1$.

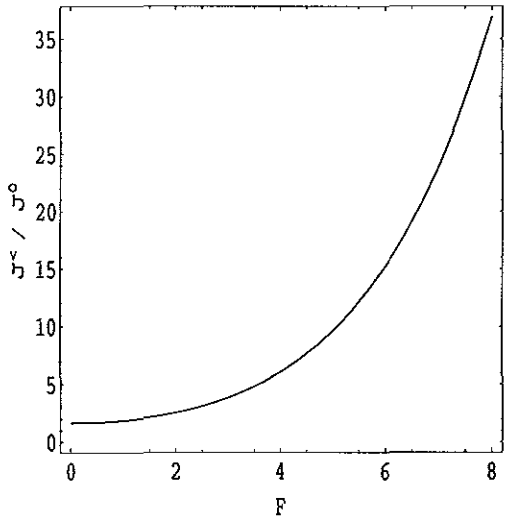


Figure 3.12: Plot of the ratio of the outward flux to the flux in the with no load and cytoplasmic concentration versus load, for $L_1 = 0.3$, $L_2 = 0.432$, $Q = 8$, and $L = 1$.

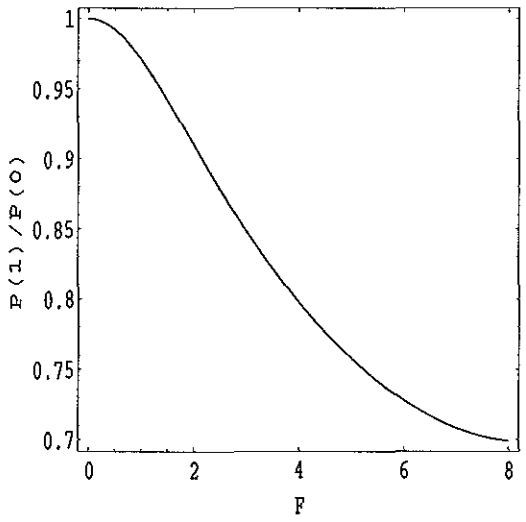


Figure 3.13: Plot of the ratio of cytoplasmic concentration to periplasmic concentration in the absence of net flux for $L_1 = 0.3$, $L_2 = 0.4$, $Q = 8$, and $L = 1$.

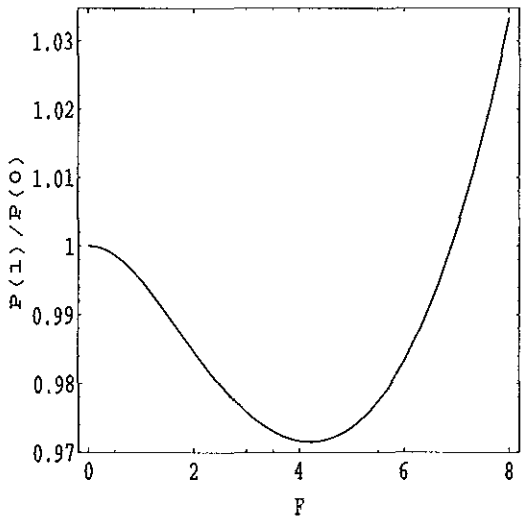


Figure 3.14: Plot of the ratio of cytoplasmic concentration to periplasmic concentration in the absence of net flux for $L_1 = 0.3$, $L_2 = 0.432$, $Q = 8$, and $L = 1$.

Chapter 4

Summary and Conclusion

We have presented a model potential of GlpF and calculated the conduction of glycerol through the channel analytically. The model we used approximates the PMF of GlpF which is constructed by SMD simulations. The flux through the channel has been calculated by taking different cases of the external load and the results are compared with those obtained numerically. Due to the action of the enzyme Glycerol Kinase inside the cell and an attractive potential well on the periplasmic side a concentration gradient is developed along the channel. In these conditions, in the zero load case, the net flux depends on the concentration gradient not on the asymmetry of the potential. This implies that, in order to produce directed transport of glycerol molecules through GlpF one needs to drive the system out of equilibrium, e.g, by applying an external force or by subjecting the system to nonequilibrium fluctuations. In the presence of periodically switching load, the net flux, inward flux, and outward flux have been compared with the flux in the case of zero load and no cytoplasmic concentration. The results are in good agreement with the numerical results of Schulten and Kosztin [7], i.e., for $L_1 = 0.3$ and $L_2 = 0.4$ the outward flux is bigger than the inward one showing that this model potential of GlpF can transport glycerol to the cell and also protects the cell against excess concentration of glycerol. The ratio of the populations at the ends of the channel is found to be in good agreement with numerical results,

suggesting that the cytoplasmic glycerol concentration should be smaller than the periplasmic glycerol concentration. The structure of GlpF exhibits a hydrophilic vestibule on the periplasmic side of the glycerol conduction pathway. This structural asymmetry is reflected in an attractive well in the potential of the ratchet. The clogging effect of the potential well on the periplasmic side is shown by comparing the fluxes through the normal and inverted channels. The ratchet potential we used to study the conduction of glycerol through GlpF approximates the actual potential (PMF). This model potential of GlpF can transport glycerol to the cell and protects the cell against excess doses of glycerol which causes cell poisoning and death.

Much of the earlier works on glycerol conduction are carried out numerically using the PMF of GlpF. Simplified models have been employed; the six steps model and the two steps model. In this paper we devised a model potential (ratchet potential) and studied the conduction of glycerol through GlpF analytically. Further works can be carried out using this model, and comparisons can be made with the six steps model and the two steps model.

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