Transit Time Distribution for Biochemical Networks Far from Equilibrium: Amplification of the Probability of Net Transformation Due to Multiple Reflections

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We investigate a nonequilibrium biochemical network described in terms of transit time distributions. We assume that the network is made up of two subsystems which are connected to each other as well as to the environment. We evaluate the probability distribution of the time necessary for a molecular species to cross the system, i.e., the distribution of the time that elapses from the moment the species enters one subsystem and leaves it by passing through the second subsystem. For this process the total probability of crossing the system is at the same time the probability of net transformation of a chemical in a desired product. The analysis leads to the surprising result that the probability of crossing the system increases with the average time spent by the species in one subsystem. A physical explanation of this apparent paradox is given by taking into account the multiple reflections occurring within the system. The escape from the system takes place after a random number of forward and backward transitions between the two subsystems. Although large transit times for the two subsystems correspond to very small individual crossing probabilities, they also favor a large number of reflections. The total crossing probability is made up by the sum of the contributions of the passage events taking place after different numbers of reflections. In this sum the low values of the different terms are outweighed by the large number of terms, resulting in an amplification of the efficiency of the passage process due to multiple reflections. The increase of the crossing probability takes place at the expense of slowing down the process. The average crossing time of the whole system increases linearly with the number of reflection events within the system. It is shown that such a phenomenon of amplification of the crossing probability may also occur in the case of thermally activated passage over a succession of energy barriers.

1. Introduction

Recently a number of interesting stochastic phenomena in nonequilibrium chemical systems have attracted attention, including stochastic resonance,[1−4] stochastic hysteresis,[5] resonant activation,[6−8] and stochastic linearization.[9,10] The purpose of the present article is to point out the existence of an effect belonging to the same class of phenomena, the amplification of the probability of crossing through a biochemical network far from equilibrium due to multiple reflections. The possible occurrence of this type of phenomenon has been suggested by some analogies reported in the literature, between complex reaction networks, neural networks, and systems of logical gates in general.[11−18] The study of such processes is of interest in biochemistry where the crossing probability expresses the probability of net transformation of a chemical to a desired product. This phenomenon, to be investigated, occurs in an open biochemical system made up of two interacting subsystems. Although the two subsystems need not be identical, for simplicity we first investigate the phenomenon in the case of symmetric systems, made up of two identical subsystems, and then we generalize the results for asymmetric systems. The plan of the paper is as follows. In section 2 the simplified symmetric system to be studied is defined. In section 3 the crossing

probability over a succession of two coupled biochemical networks is evaluated by applying the method of age-dependent master equations. In section 4 a physical interpretation of the solutions of the age-dependent master equations is given in terms of the multiple reflections occurring within the system. In section 5 the theory is extended to asymmetric systems. Section 6 deals with the numerical analysis of the process of amplification due to multiple reflections in the case of a simple biochemical network. In section 7 we present an analogy between the biochemical system studied in this paper and the thermally activated passage over a succession of energy barriers. Finally, in section 8 the general implications of the amplification of crossing probability due to multiple reflections are investigated.

2. Formulation of the Problem

Our approach is guided by our recent study of biochemical networks in terms of transit times.[19,20] This theory is based on a random variable, the *transit time θ*, which is defined as the age of (metabolic) intermediates at the time of leaving the system. The theory, based on a semistochastic approach, leads to calculations of the probability distributions of the ages of the intermediates, as functions of time. By assuming that the kinetics of the pathway is described by mass-action laws, a system of partial differential equations is derived for the distribution function of the transit time. An attractive feature of this approach is that for certain systems it leads to a system

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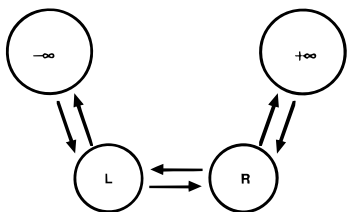§ Universidad Complutense de Madrid.

Figure 1. Graphical representation of a symmetric biochemical system interacting with the environment. The system is made up of two different identical subsystems, L and R, which exchange chemicals with each other as well as with the environment. We assume, for ease of computational purposes, that the environment can be also divided in two compartments, denoted by $-\infty$ and $+\infty$, respectively.

of integrodifferential evolution equations for the age distributions, which are amenable to analytical treatment, provided that the numeric or analytic solution of the mass-action equations is known.

In the following we consider a biochemical network made up from two identical subsystems which are connected to each other as well as to the environment (see Figure 1). There are two types of reaction intermediates: (a) main intermediates, denoted by X_j, $j = 1, ..., n$, which can be either free intermediates I_u, $u = 1, 2, ...$, or bound intermediates $I_u E_v$, u, $v = 1, 2, ...$, where E_v, $v = 1, 2, ...$, are different enzymes; (b) other chemicals, denoted by A_j, $j = 1, 2, ...$; this second group includes the free enzymes E_v, $v = 1, 2, ...$, as well as other chemicals which may be present in the system. We assume that the reactions involving the main intermediates X_j, $j = 1, ..., n$, have the following structure:

$$... + X_j \underset{k_{j'j}(\mathbf{A})}{\overset{k_{jj'}(\mathbf{A})}{\rightleftarrows}} X_{j'} + ... \qquad n \geq j' \geq j \geq 1 \qquad (1)$$
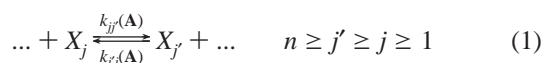
where $(... +$ and $+ ... +)$ denotes the contribution of the other chemicals A_j, $j = 1, 2, ...$ to the process and $k_{jj'}(\mathbf{A})$, $k_{j'j}(\mathbf{A})$ are effective rate coefficients depending on the composition vector $\mathbf{A}$ of the concentrations A_1, A_2, ... of the other chemicals ($\mathbf{A} = (A_1, A_2, ...)$). In addition to the processes (1), the intermediates X_j, $j = 1, ..., n$, are involved in two other types of processes: (a) They are generated with different rates $J_1(t), ..., J_n(t)$ which are generally time dependent:

$$... \underset{k_j^{\#}(\mathbf{A})}{\overset{J_j(t)}{\rightleftarrows}} X_j, \qquad j = 1, ..., n \qquad (2)$$
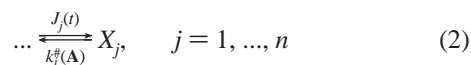
(b) They interact with the other chemicals present in the system and generate different final products $P_1, ..., P_n$

$$... + X_j \underset{J_j(t)}{\overset{k_j^*(\mathbf{A})}{\rightleftarrows}} P_j, \qquad j = 1, ..., n \qquad (3)$$
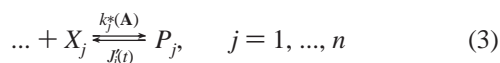
where $k_j^*(\mathbf{A})$, $j = 1, ..., n$, are effective rate coefficients. Thus, all reactions involving each intermediate are linear in that intermediate. However, the overall kinetics of the process is nonlinear.

For simplicity, in this article we confine ourselves to the case when the system is operated in a stationary regime; that is, we assume that the fluxes $J_1(t), ..., J_n(t)$ are time independent and that the kinetic equations corresponding to the reactions 1–3 have stationary solutions. It has been shown in ref 19 that, under these circumstances, it is possible to derive a system of partial differential equations for the distributions of transit times.

In Figure 1 are represented two identical subsystems, **L** and **R**, which interact with each other as well as with the environ-

ment. In order to simplify the computations, we assume that the environment can be also divided in two compartments, denoted by $-\infty$ and $+\infty$, respectively. For the system displayed in Figure 1 it is possible to introduce the many distribution functions of the transit time. We denote by $\psi_e(\theta)d\theta$ the probability density of the transit time of a chemical species which leaves the system without crossing both subsystems; such a particle enters a given subsystem from the environment, stays in that subsystem for a time interval between θ and $\theta + d\theta$ and then returns to the environment. We also introduce the individual crossing probability $\psi_s(\theta) \, d\theta$ that a species leaves one subsystem after a time interval between θ and $\theta + d\theta$ and enters the other subsystem. These two probability densities obey the normalization condition

$$\int_0^\infty \psi_e(\theta) \, d\theta + \int_0^\infty \psi_s(\theta) \, d\theta = 1 \qquad (4)$$

and can be evaluated from the kinetic parameters of the network 1–3 by using the method presented in refs 19 and 20. Although $\psi_e(\theta) \, d\theta$ and $\psi_s(\theta) \, d\theta$ characterize only the individual crossings, from one subsystem to the environment, and from one to the other subsystem, they contain the whole information necessary for the determination of the stochastic properties of an arbitrary succession of multiple crossings. In the following, starting out from the set of functions $\psi_e(\theta) \, d\theta$ and $\psi_s(\theta) \, d\theta$, we evaluate the total probability that a species leaves the system after passing at least once through both subsystems.

3. Evolution Equations

In terms of the transit time distributions $\psi_e(\theta) \, d\theta$ and $\psi_s(\theta) \, d\theta$, we can compute the survival probability $l(\theta)$ of the species in the system after a waiting time interval θ

$$l(\theta) = \int_\theta^\infty \psi_e(\theta') \, d\theta' + \int_\theta^\infty \psi_s(\theta') \, d\theta' \qquad (5)$$

that is, the probability that in a waiting time interval of length θ the species stays in the system. In terms of the three functions $\psi_e(\theta) \, d\theta$, $\psi_s(\theta) \, d\theta$, and $l(\theta)$, we can introduce the individual crossing rates:

$$g_{e,s}(\theta) = \psi_{e,s}(\theta)/l(\theta) \qquad (6)$$

Here $g_e(\theta)$ is the rate of chemical species crossing from a subsystem to the environment after a waiting time between θ and $\theta + d\theta$, whereas $g_s(\theta)$ is the rate of transition from one subsystem to the other subsystem after a waiting time between θ and $\theta + d\theta$. The rates $g_{e,s}(\theta)$ determine completely the stochastic properties of the transit time. In particular, from eqs 4–6 it is easy to show that we have (see Appendix):

$$\psi_{e,s}(\theta) = g_{e,s}(\theta) \exp[-\int_0^\theta g_e(\theta') \, d\theta' - \int_0^\theta g_s(\theta') \, d\theta'] \qquad (7)$$

As the rates $g_{e,s}(\theta)$ are generally dependent on the waiting time θ, that is on the time interval between two crossings, the corresponding random process is semi-Markovian and can be described by using the method of age-dependent master equations (ADME[21]). We define the following probability densities and probabilities:

$\mathcal{P}_{L,R}(\theta; t) \, d\theta$: the probability that at time t a species is in the subsystem u, where $u = L, R$ and that the residence time in the subsystem $u = L, R$ is between θ and $\theta + d\theta$.

$P_{\pm\infty}(t)$: the probability that at time t the species is out of the system in the region u, where $u = \pm\infty$. These probability densities fulfill the normalization condition:

Transit Time Distribution for Biochemical Networks

J. Phys. Chem. B, Vol. 103, No. 19, 1999 **3967**

$$\int_0^\infty [\mathcal{P}_L(\theta; t) + \mathcal{P}_R(\theta; t)] \, d\theta + P_{-\infty}(t) + P_{+\infty}(t) = 1 \quad (8)$$

By applying the ADME approach[21] we can derive the following system of integrodifferential equations for $\mathcal{P}_{L;R}(\theta; t) \, d\theta$ and $P_{\pm\infty}(t)$:

$$\left(\frac{\partial}{\partial t} + \frac{\partial}{\partial \theta}\right)\mathcal{P}_{L;R}(\theta; t) + [g_s(\theta) + g_e(\theta)]\mathcal{P}_{L;R}(\theta; t) = 0 \quad (9)$$

$$\mathcal{P}_{L;R}(\theta = 0; t) = \int_0^\infty \mathcal{P}_{R;L}(\theta'; t)g_s(\theta') \, d\theta' \quad (10)$$

$$\frac{d}{dt} P_{\pm\infty}(t) = \int_0^\infty \mathcal{P}_{R;L}(\theta'; t)g_e(\theta') \, d\theta' \quad (11)$$

with the initial conditions

$$\mathcal{P}_{L;R}(\theta; t=0) = p_{L;R}^* \delta(\theta) \quad (12)$$

$$P_{\pm\infty}(t=0) = 0 \quad (13)$$

where p_L^* and $p_R^* = 1 - p_L^*$ are the probabilities that initially the particle is in the subsystem L or R, respectively. We have assumed that we start studying the process from the moment one species entered the system. Moreover, we have considered that at $t = 0$ the species is either in the subsystem L with a probability p_L^* or in the subsystem R, with a probability $p_R^* = 1 - p_L^*$.

Equations 9 can be integrated along the characteristics; the corresponding solutions depend on the boundary values $\mathcal{P}_{L;R}(\theta=0;t)$. By inserting the solutions for $\mathcal{P}_{L;R}(\theta;t)$ into eqs 10 we get two integral equations for $\mathcal{P}_{L;R}(\theta=0;t)$ which can be solved by using the method of Laplace transformation. By combining the expressions for $\mathcal{P}_{L;R}(\theta;t)$ and $\mathcal{P}_{L;R}(\theta=0;t)$ with eqs 7 and 11 we can compute the probabilities $P_{\pm\infty}(t)$:

$$P_{\pm\infty}(t) = \mathcal{L}^{-1}\left\{\frac{[\bar{\psi}_s(\sigma)p_{L;R}^* + p_{R;L}^*]\bar{\psi}_e(\sigma)}{\sigma[1 - (\bar{\psi}_s(\sigma))^2]}\right\} \quad (14)$$

where

$$\bar{\psi}_{e,s}(\sigma) = \int_0^\infty \exp(-\theta\sigma)\psi_{e,s}(\theta) \, d\theta \quad (15)$$

are the Laplace transforms of $\psi_{e,s}(\theta)$, σ is the Laplace variable conjugate to the time and the waiting time, and $\mathcal{L}^{-1}$ denotes the inverse Laplace transformation. The derivation of eq 14 is lengthy but standard; to save space the intermediate steps are not given here.

The probability densities $\Pi_{L;R}(t)$ of the crossing time of the particle from the system to the exterior are given by

$$\Pi_{L;R}(t) = \frac{dP_{\mp\infty}(t)}{dt} = \mathcal{L}^{-1}\left\{\frac{[\bar{\psi}_s(\sigma)p_{R;L}^* + p_{L;R}^*]\bar{\psi}_e(\sigma)}{1 - (\bar{\psi}_s(\sigma))^2}\right\} \quad (16)$$

Here $\Pi_{L;R}(t)$ are the probability densities of the crossing time of the species from the system to the environment from the left (L) or the right (R) subsystem, respectively. It is easy to check that the probability densities $\Pi_{L;R}(t)$ given by eqs 16 are properly normalized; that is, they obey the normalization condition:

$$\int_0^\infty [\Pi_L(t) + \Pi_R(t)] \, dt = 1 \quad (17)$$

We are especially interested in the evaluation of the total crossing probability T_2 of the two subsystems, L and R.

Considering a succession of transitions from L to R, we have

$$T_2 = \int_0^\infty \Pi_R(t|p_L^* = 1, p_R^* = 0) \, dt \quad (18)$$

Similarly, for a succession of transitions from R to L we obtain

$$T_2 = \int_0^\infty \Pi_L(t|p_L^* = 0, p_R^* = 1) \, dt \quad (19)$$

Due to the symmetry of the system the total crossing probabilities T_2 given by eqs 18 and 19 should be the same. By applying eqs 16 and 18−19 we obtain

$$T_2 = \int_0^\infty \Pi_L(t|p_L^* = 0, p_R^* = 1) \, dt = \angle[\Pi_L(t|p_L^* = 0, p_R^* =$$

$$1)]|_{\sigma=0} = \frac{[\bar{\psi}_s(\sigma=0)p_{R;L}^* + p_{L;R}^*]\bar{\psi}_e(\sigma=0)}{1 - (\bar{\psi}_s(\sigma=0))^2} = \alpha/(1 + \alpha) \quad (20)$$

where

$$\alpha = \int_0^\infty \psi_s(\theta) \, d\theta \quad (21)$$

is the probability for the passage from one subsystem to the other subsystem. The complementary probability

$$\beta = 1 - \alpha = \int_0^\infty \psi_e(\theta) \, d\theta \quad (22)$$

is the total probability of an individual crossing from one subsystem to the environment. The ratio of the two probabilities

$$\epsilon = \alpha/\beta = \int_0^\infty \psi_s(\theta)d\theta / \int_0^\infty \psi_e(\theta) \, d\theta \quad (23)$$

is a measure of the number of passage events from one subsystem to another corresponding to one individual exit event from one subsystem to the environment. At first sight it seems that the crossing probability T_2 should be large when the number of exit events is much larger than the number of passages from one subsystem to another, that is, for $\epsilon \gg 1$, and small in the other situation when the number of intersystem transitions is much larger than the number of exits, i.e., for $\epsilon \ll 1$. However, eq 20 predicts exactly the opposite, i.e., that T_2 increases monotonically from $T_2 = 0$ for $\epsilon = 0$ to the asymptotic value $1/2$ for $\epsilon \to \infty$. We have

$$T_2 = \epsilon/(2\epsilon + 1) \sim \begin{cases} \epsilon & \text{for } \epsilon \ll 1 \\ 1/2 & \text{for } \epsilon \gg 1 \end{cases} \quad (24)$$

The explanation of this apparent paradox is the subject of the following section.

4. Amplification of Crossing Probability by Multiple Reflections

In order to give a physical explanation of the behavior of the crossing probability T_2 given by eq 24, we try to give a physical derivation of eqs 16 and 20 by taking into account all kinds of reflection and crossing processes which can take place within the system. This method is based on the technique of continuous time random walks (CTRW[22]). We illustrate the technique by computing the probability density $\Pi_L(t)$ of crossing the system in the order R → L → −∞. $\Pi_L(t)$ is made up of two contributions

$$\Pi_L(t) = \Pi_L^{(1)}(t) + \Pi_L^{(2)}(t) \quad (25)$$

3968 *J. Phys. Chem. B, Vol. 103, No. 19, 1999*

Vlad et al.

where $\Pi_L^{(1)}(t)$ and $\Pi_L^{(2)}(t)$ are the contributions corresponding to the cases when the species is initially placed in the subsystem L and R, respectively. $\Pi_L^{(1)}(t)$ is made up of the contribution of the direct crossing $L \to -\infty$ as well as by the contributions of the crossings taking place after different numbers of reflections in the system. We have

$$\Pi_L^{(1)}(t) = p_1^*[\psi_e(t) + \sum_{q=1}^{\infty} [\psi_s(t)\otimes]^{(2q)} \otimes \psi_e(t)] \quad (26)$$

where $\otimes$ denotes the temporal convolution product. The different terms in eq 26 correspond to the transitions

$$L \to -\infty; L \to R \to L \to -\infty, ..., L \to (R \to L)_{q \text{ times}} \to$$
$$-\infty, ... \quad (27)$$

$\Pi_L^{(2)}(t)$ is given by a similar relation

$$\Pi_L^{(2)}(t) = p_2^*[\psi_s(t)\otimes\psi_e(t) + \sum_{q=0}^{\infty} [\psi_s(t)\otimes]^{(2q+1)}\otimes\psi_e(t)] \quad (28)$$

which expresses the contributions of the transitions

$$R \to L \to -\infty; R \to L \to R \to L \to -\infty, ...,$$
$$(R \to L)_{(q+1) \text{ times}} \to -\infty, ... \quad (27)$$

By applying the Laplace transformation to eqs 25, 26, and 28, performing a formal summation of the resulting geometric series, and coming back to the time variable, we recover the first of the two eqs 16. The second eq 16 for $\Pi_R(t)$ can be rederived in a similar way.

The total crossing probability T_2 for the succession of the two subsystems can be also computed by taking the different types of reflections into account. For example, the analogue of eq 19 for the crossing of the system in the succession $R \to L \to -\infty$ is

$$T_2 = \alpha\beta + \sum_{q=0}^{\infty} \alpha^{2q+1}\beta = \alpha/(1 + \alpha) \quad (30)$$

which expresses the contribution of the direct crossing $R \to L \to -\infty$ as well as of the indirect crossings occurring after different numbers of reflections. The series expansion (30) allows us to establish the validity range of eq 20. The geometric series (30) converges provided that $1 > \alpha \geq 0$. In this case from eq 25 we recover eq 20 for the crossing factor T_2. For $\alpha \to 1$ the series in eq 30 diverges, the probability β tends to zero, $\beta \to 0$, and the expression 17 for T_2 is no longer valid. In this case we have

$$T_2(\alpha=1) = 0 \quad (31)$$

Equation 30 shows clearly that the apparent paradox pointed out in the preceding section is caused by the multiple reflections of the molecular species within the system. A large value of α, very close to but different from unity, leads to a very low value of the probability $\beta = 1 - \alpha$ of the direct crossing from the system to the environment; however, it also favors a large number of reflections. The probability of occurrence of a number q of reflection pairs is given by a Pascal distribution

$$\eta_q = (1 - \alpha^2)\alpha^{2q} \quad (32)$$

and thus the average number of reflection pairs is equal to

$$\langle q \rangle = \sum_{q=0}^{\infty} q\eta_q = \alpha^2/(1 - \alpha^2) \quad (33)$$

For low α the average number of reflection pairs is close to zero; however, as α approaches unity, $\alpha \to 1$, the mean number of reflection pairs diverges to infinity. Due to this increase in the number of reflections, the decrease of the individual probability $\beta = 1 - \alpha$ as $\alpha \to 1$ is compensated and even outweighed by the large number of crossings occurring after different numbers of reflections. It follows that multiple reflections within the system increase the probability of occurrence of the overall crossing process of the two subsystems, resulting in large values of the probability T_2. In order that this amplification mechanism by multiple reflections be efficient, it is necessary that the probability α is very close to, but different from, unity. In this case the probability T_2 is made up of a very large number of contributions each of them being very small. However, if α is exactly equal to unity, even though the average number of reflections becomes infinite, because the probability $\beta = 1 - \alpha$ of a direct crossing to the environment is exactly equal to zero, the amplification mechanism does not work anymore, the particle is trapped forever in the system, and the overall crossing factor T_2 is equal to zero (see eq 31).

For a deeper understanding of the amplification of the crossing probability due to multiple reflections, we compute the average time necessary for crossing the system. We normalize the probability density $\Pi_R(t|p_L^* = 1, p_R^* = 0) = \Pi_L(t|p_L^* = 0, p_R^* = 1)$ by dividing it by the overall crossing factor T_2:

$$\Phi(t) = \Pi_R(t|p_L^* = 1, p_R^* = 0)/T_2 = \Pi_L(t|p_L^* = 0, p_R^* = 1)/T_2 = \mathcal{L}^{-1}\left\{\frac{(1 - \alpha^2)\bar{\varphi}_e(\sigma)\bar{\varphi}_s(\sigma)}{1 - \alpha^2(\bar{\varphi}_s(\sigma))^2}\right\} \quad (34)$$

where

$$\Phi(t)\,\mathrm{d}t \qquad \text{with } \int_0^{\infty} \Phi(t)\,\mathrm{d}t = 1 \quad (35)$$

is the overall probability that the time necessary for crossing the system is between t and $t + \mathrm{d}t$;

$$\bar{\varphi}_{e,s}(\sigma) = \int_0^{\infty} \exp(-\sigma\theta)\varphi_{e,s}(\theta)\,\mathrm{d}\theta \quad (36)$$

are the Laplace transforms of the normalized probability densities

$$\varphi_e(\theta) = \psi_e(\theta)/\int_0^{\infty} \psi_e(\theta)\,\mathrm{d}\theta = \psi_e(\theta)/(1 - \alpha)$$
$$\text{with } \int_0^{\infty} \varphi_e(\theta)\,\mathrm{d}\theta = 1 \quad (37)$$

$$\varphi_s(\theta) = \psi_s(\theta)/\int_0^{\infty} \psi_s(\theta)\,\mathrm{d}\theta = \psi_s(\theta)/\alpha$$
$$\text{with } \int_0^{\infty} \varphi_s(\theta)\,\mathrm{d}\theta = 1 \quad (38)$$

of the individual escape time and of the passage time from one subsystem to another, respectively.

For evaluating the moments of the overall crossing time

$$\langle t^m \rangle = \int_0^{\infty} t^m \Phi(t)\,\mathrm{d}t, \quad m = 1, 2, ..., \quad (39)$$

we expand the Laplace transform of the probability density $\Phi(t)$ in a Taylor series, resulting in

$$\bar{\Phi}(\sigma) = \int_0^\infty \exp(-\sigma\theta)\Phi(\theta)\,\mathrm{d}\theta = 1 + \sum_{m=1}^\infty \frac{(-1)^m}{m!}\langle t^m\rangle\sigma^m =$$

$$\frac{(1-\alpha^2)\bar{\varphi}_e(\sigma)\bar{\varphi}_s(\sigma)}{1-\alpha^2(\bar{\varphi}_s(\sigma))^2} \quad (40)$$

In particular, the first term of the expansion gives

$$\langle t\rangle = -\frac{\mathrm{d}\bar{\Phi}(\sigma=0)}{\mathrm{d}\sigma} = \langle\theta\rangle_e + \langle\theta\rangle_s\left[\frac{1+\alpha^2}{1-a^2}\right] \quad (41)$$

where

$$\langle\theta\rangle_{e,s} = \int_0^\infty \theta\varphi_{e,s}(\theta)\,\mathrm{d}\theta = -\frac{\mathrm{d}\bar{\varphi}_{e,s}(\sigma=0)}{\mathrm{d}\sigma} \quad (42)$$

are the average individual escape time and the average passage time from one subsystem to another, respectively.

From eq 41 we note that the total escape time monotonically increases from $\langle\theta\rangle_e + \langle\theta\rangle_s$ for $\alpha = 0$ (no multiple reflections) to infinity for $\alpha = 1$. It follows that the increase of the probability of crossing takes place at the expense of an increase of the average escape time. This time increase is due to the additive contributions of the individual pieces of time necessary for the occurrence of the individual reflections. On the basis of this observation we can give a physical derivation of eq 41 for the average crossing time similar to the one given in section 4 for eqs 16 and 20, for the probability densities $\Pi_{L;R}(t)$ of the crossing time and for the crossing probability T_2, respectively. By taking into account that a simple crossing requires an average time $\langle\theta\rangle_e + \langle\theta\rangle_s$ and that each pair of reflections requires an average time $2\langle\theta\rangle_s$ we simply have

$$\langle t\rangle = \langle\theta\rangle_e + \langle\theta\rangle_s + 2\langle\theta\rangle_s\langle q\rangle \quad (43)$$

from which, by using eq 33 for the average number $\langle q\rangle$ of reflections we recover eq 41.

5. Asymmetric Systems

The amplification phenomenon due to multiple reflections also occurs in the case when the two interacting subsystems are not identical. The theory developed in the preceding sections can be easily extended to asymmetric systems. The main difference is that the probabilities $\psi_e(\theta)\,\mathrm{d}\theta$ and $\psi_s(\theta)\,\mathrm{d}\theta$ of the individual crossing events are different for the two subsystems. To show this we identify them by superscript labels which show that the corresponding probability refers to a given subsystem. We have four probabilities, $\psi_e^{(L)}(\theta)\,\mathrm{d}\theta$, $\psi_e^{(R)}(\theta)\,\mathrm{d}\theta$, $\psi_s^{(L)}(\theta)\,\mathrm{d}\theta$, and $\psi_s^{(R)}(\theta)\,\mathrm{d}\theta$ which fulfill two different normalization conditions

$$\int_0^\infty \psi_e^{(L)}(\theta)\,\mathrm{d}\theta + \int_0^\infty \psi_s^{(L)}(\theta)\,\mathrm{d}\theta = 1, \int_0^\infty \psi_e^{(R)}(\theta)\,\mathrm{d}\theta + \int_0^\infty \psi_s^{(R)}(\theta)\,\mathrm{d}\theta = 1 \quad (44,45)$$

The probability densities $\Pi_{L;R}(t)$ of the crossing time of the particle from the system to the exterior are equal to

$$\Pi_{L;R}(t) = \frac{\mathrm{d}P_{\mp\infty}(t)}{\mathrm{d}t} = \angle^{-1}\left\{\frac{[\bar{\psi}_s^{(R;L)}(\sigma)p_{R;L}^* + p_{L;R}^*]\bar{\psi}_e^{(L;R)}(\sigma)}{1-\bar{\psi}_s^{(L)}(\sigma)\bar{\psi}_s^{(R)}(\sigma)}\right\} \quad (46)$$

and the total crossing probability $T_2^{(L;R)}$ can be evaluated by

using eqs 18 and 19, resulting in

$$T_2^{(L;R)} = \frac{\alpha_{L;R}(1-\alpha_{R;L})}{1-\alpha_L\alpha_R} \quad (47)$$

where:

$$\alpha_{L;R} = \int_0^\infty \psi_s^{(L;R)}(\theta)\,\mathrm{d}\theta \quad (48)$$

In the asymmetric case the overall probability

$$\Phi^{(L;R)}(t)\,\mathrm{d}t, \quad\text{with } \int_0^\infty \Phi^{(L;R)}(t)\,\mathrm{d}t = 1 \quad (49)$$

depends on the subsystem considered. We have

$$\Phi^{(L;R)}(t) = \angle^{-1}\left\{\frac{(1-\alpha_L\alpha_R)\bar{\varphi}_e^{(L;R)}(\sigma)\bar{\varphi}_s^{(R;L)}(\sigma)}{1-\alpha_L\alpha_R\bar{\varphi}_s^{(L)}(\sigma)\bar{\varphi}_s^{(R)}(\sigma)}\right\} \quad (49a)$$

where

$$\bar{\varphi}_{e;s}^{(L;R)}(\sigma) = \int_0^\infty \exp(-\theta\sigma)\varphi_{e;s}^{(L;R)}(\theta)\,\mathrm{d}\theta \quad (50)$$

are the Laplace transforms of the normalized probability densities:

$$\varphi_{e;s}^{(L;R)}(\theta) = \psi_{e;s}^{(L;R)}(\theta)/\int_0^\infty \psi_{e;s}^{(L;R)}(\theta)\,\mathrm{d}\theta,$$
$$\text{with } \int_0^\infty \varphi_{e;s}^{(L;R)}(\theta)\,\mathrm{d}\theta = 1 \quad (51)$$

The expression 41 for the average escape time becomes

$$\langle t\rangle^{(L;R)} = \int_0^\infty t\Phi^{(L;R)}(t)\,\mathrm{d}t = \langle\theta\rangle_e^{(L;R)} + \langle\theta\rangle_s^{(R;L)} +$$
$$\frac{\alpha_L\alpha_R}{1-\alpha_L\alpha_R}[\langle\theta\rangle_s^{(L)} + \langle\theta\rangle_s^{(R)}] \quad (52)$$

where the individual escape times $\langle\theta\rangle_{e;s}^{(L;R)}$ are given by

$$\langle\theta\rangle_{e;s}^{(L;R)} = \int_0^\infty \theta\varphi_{e;s}^{(L;R)}(\theta)\,\mathrm{d}\theta \quad (53)$$

Equation 52 has a physical interpretation similar to the one of eq 41. The sum $\langle\theta\rangle_s^{(L)} + \langle\theta\rangle_s^{(R)}$ is the average time interval necessary for the occurrence of a reflection pair. If we multiply this time interval by the average number $\langle q\rangle$ of the reflection pairs in the system, and add this to the result that the average time necessary for the occurrence of a simple crossing is $\langle\theta\rangle_e^{(L;R)} + \langle\theta\rangle_s^{(R;L)}$, we obtain

$$\langle t\rangle^{(L;R)} = \langle\theta\rangle_e^{(L;R)} + \langle\theta\rangle_s^{(R;L)} + \langle q\rangle[\langle\theta\rangle_s^{(L)} + \langle\theta\rangle_s^{(R)}] \quad (54)$$

Since the probability η_q of the occurrence of a number q of reflection pairs is given by

$$\eta_q = (1-\alpha_L\alpha_R)(\alpha_L\alpha_R)^q \quad (55)$$

we obtain

$$\langle q\rangle = \sum_{q=0}^\infty q\eta_q = (\alpha_L\alpha_R)/(1-\alpha_L\alpha_R) \quad (56)$$
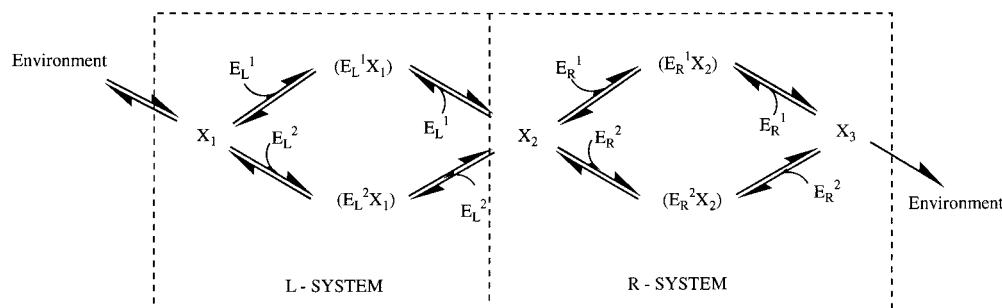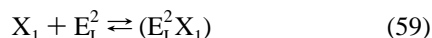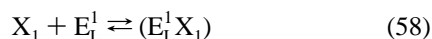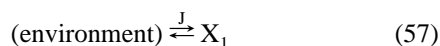
Figure 2. Biochemical model used as an example. It represents the transformation network of substrate X_1 into product X_3 through the intermediate X_2 by means of two enzyme systems composed of two isoenzymes each. Each subsystem is indicated by a dotted square.

Equations 54 and 56 lead to eq 52.

6. Biochemical Example

The application of the general theory for asymmetric systems is illustrated through a simple biochemical network (see Figure 2). We assume that a substrate X_1 is transformed into a reaction intermediate X_2 due to the catalytic action of two isoenzymes E_L^1 and E_L^2. Further on, the reaction intermediate X_2 is also transformed by two isoenzymes E_R^1 and E_R^2 into a reaction product X_3. In this example the two subsystems correspond to the reactions involving the two isoenzymes E_L and the two isoenzymes E_R, respectively. We assume that each subnetwork is made up by the two steps involving the different enzyme-substrate complexes $(E_L^1 X_1)$, $(E_L^2 X_1)$, and $(E_R^1 X_2)$, $(E_R^2 X_2)$, respectively. The reactions corresponding to the subsystem L are

$$\text{(environment)} \underset{}{\overset{J}{\rightleftarrows}} X_1 \tag{57}$$

$$X_1 + E_L^1 \rightleftarrows (E_L^1 X_1) \tag{58}$$

$$X_1 + E_L^2 \rightleftarrows (E_L^2 X_1) \tag{59}$$

The connection between the two subsystems is made by the two reactions

$$(E_L^1 X_1) \rightleftarrows X_2 + E_L^1 \tag{60}$$

$$(E_L^2 X_1) \rightleftarrows X_2 + E_L^2 \tag{61}$$
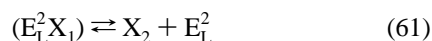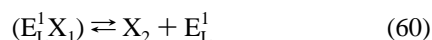
giving the intermediate X_2. The reactions corresponding to the second subsystem, R, are

$$X_2 + E_L^1 \rightleftarrows (E_R^1 X_2) \tag{62}$$

$$X_2 + E_R^2 \rightleftarrows (E_R^2 X_2) \tag{63}$$
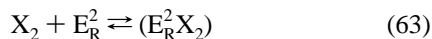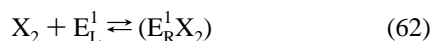
The connection between the R subsystem and the environment is made by the two reactions that generate the reaction product X_3:

$$(E_R^1 X_2) \rightleftarrows X_3 + E_R^1 \tag{64}$$

$$(E_R^2 X_2) \rightleftarrows X_3 + E_R^2 \tag{65}$$
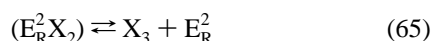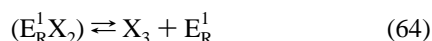
Finally, the reaction product leaves the system. This process can be represented by an irreversible step:

$$X_3 \rightarrow \text{(environment)} \tag{66}$$
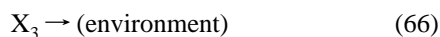
We assume that the system is maintained in a stationary state by a constant influx J_1 of the substrate X_1. The possible existence of a stationary regime for the system considered seems reasonable, although there is no warranty that it exists for any values of the reaction parameters. We are interested in checking the existence of the amplification of the probability of net transformation due to multiple reflections for this system. For a system operated in a stationary regime we follow the evolution of a molecule X_1 from the influx J_1 and try to evaluate the stochastic properties of the time necessary for the transformation of this molecule X_1 into a molecule of reaction product X_3.

For this particular system the biochemical interpretation of the ideas introduced in the preceding sections is the following. A chemical enters the L-subsystem from the environment, is transformed into the intermediate X_1, and then through a chain of transformations crosses to the system R as the intermediate X_2. In the R-subsystem the intermediate enters a chain of other reactions and is eventually transformed into X_3 and leaves the system. This chain of transformations does not have 100% yield: a certain fraction of the initial chemical is not transformed into the final product and returns unchanged to the environment. In this case the crossing time is the time necessary for the transformation of the initial chemical into the final product and the probability distribution $\Pi_{L;R}(t)$ dt of the crossing time can be interpreted as the probability distribution for the chemical transformation to take place. The crossing probability, $T_2^{(L)}$, is the probability of net transformation of the initial chemical to the final product and the complementary probability, $1 - T_2^{(L)}$, is the probability that the initial chemical leaves the system unchanged.

By applying the general theory developed in refs 19 and 20 it follows that the probabilities $\psi_e^{(L)}(\theta)$ dθ, $\psi_e^{(R)}(\theta)$ dθ, $\psi_s^{(L)}(\theta)$ dθ, and $\psi_s^{(R)}(\theta)$ dθ depend on the eigenvalues of a secular equation and of the corresponding eigenvectors, which depend on the properties of the system. We assume, for simplicity, that all eigenvalues are distinct. We mention that this assumption has been fulfilled in the case of our numerical calculations. In order that the stationary state considered is stable the eigenvalues must be at most equal to zero. However, because our stochastic approach takes into account the possibility of escaping from the system to the environment, there are no eigenvalues equal to zero. Under these circumstances the probabilities $\psi_e^{(L)}(\theta)$ dθ, $\psi_e^{(R)}(\theta)$ dθ, $\psi_s^{(L)}(\theta)$ dθ, and $\psi_s^{(R)}(\theta)$ dθ may be represented as

$$\psi^{(L;R)}_{e;s}(\theta) = \sum_{n \geq 1} f_{e;s}^{(L;R)(n)} \exp[-\theta \chi_n] \tag{67}$$

where $-\chi_n$ are the eigenvalues of the secular equation and thus

$$\chi_n > 0, \quad n = 1, 2, \ldots \tag{68}$$

Transit Time Distribution for Biochemical Networks

J. Phys. Chem. B, Vol. 103, No. 19, 1999 **3971**

The amplitude factors, $f_{e;s}^{(L;R)(n)}$, which depend on the eigenvectors of the kinetic matrix satisfy the following restrictions

$$\sum_n (f_e^{(L;R)(n)} + f_s^{(L;R)(n)})/\chi_n = 1 \qquad (69)$$

which ensure the validity of the normalization conditions (44) and (45).

The Laplace transform $\bar{\varphi}_{e;s}^{(L;R)}(\sigma)$ of the individual probability distributions $\varphi_{e;s}^{(L;R)} \, d\theta$ as well as the individual crossing factors α_L and α_R can be easily evaluated. After some calculations we obtain

$$\bar{\varphi}_{e;s}^{(L;R)}(\sigma) = \left[\sum_n \frac{f_{e;s}^{(L;R)(n)}}{\chi_n + \sigma}\right] \Big/ \left[\sum_n \frac{f_{e;s}^{(L;R)(n)}}{\chi_n}\right] \qquad (70)$$

$$\alpha_{L;R} = \sum_n \frac{f_s^{(L;R)(n)}}{\chi_n} \qquad (71)$$

From these equations we can also evaluate the individual escape times $\langle\theta\rangle_{e;s}^{(L;R)}$ by differentiating $\bar{\varphi}_{e;s}^{(L;R)}(\sigma)$ with respect to σ and putting $\sigma = 0$:

$$\langle\theta\rangle_{e;s}^{(L;R)} = -\frac{d}{d\sigma}\bar{\varphi}_{e;s}^{(L;R)}(\sigma=0) = \left[\sum_n \frac{f_{e;s}^{(L;R)(n)}}{(\chi_n)^2}\right] \Big/ \left[\sum_n \frac{f_{e;s}^{(L;R)(n)}}{\chi_n}\right] \qquad (72)$$

The system is kept away from equilibrium by the constant influx J of fresh substrate X_1. One molecule X_1 entering the subsystems L can have two different types of behaviors. (a) After a random number of forward and backward transformations it returns to the state X_1 and leaves the system in this form. The final stage of this type of evolution is described by the backward step of the reaction 57. (b) After undergoing a number of transformations the chemical species X_1 is transformed into the product X_3 and leaves the system. The final step of this transformation is described by reaction 66. Since the system studied transforms the chemical species X_1 into the species X_3, we are interested in the probability of occurrence of such a transformation, which, according to the theory developed in this section is given by $T_2^{(L)}$. The complementary probability, $1 - T_2^{(L)}$, expresses the possibility that a molecule X_1, after a number of different transformations within the system, is transformed back in its initial state X_1 and leaves the system. The biochemical network represented by the chemical reactions (57)−(66) is rather complex and its behavior depends on a large number of parameters: the influx rate J, 18 rate coefficients, and the four total concentrations of enzymes, E_L^1, E_L^2, E_R^1, and E_R^2, free and bounded. Out of these 23 parameters, however, only a limited number of variables have an important influence on the process of amplification. The first important variable is the influx rate J. An important role is also played by the fractions

$$\rho_{1,2} = k_L^{(1,2)}/(k_L^{(1,2)} + k_R^{(1,2)}) \qquad (73)$$

which express the relative contribution of the forward rate constants $k_L^{(1,2)}$ of the forward steps, $(E_L^{1,2} X_1) \rightarrow X_2 + E_L^{1,2}$ of reactions 60−61 and of the backward rate constant, $k_R^{(1,2)}$ of the backward steps, $X_2 + E_L^{1,2} \rightarrow (E_L^{1,2} X_1)$. The fractions $\rho_{1,2}$ are a measure of the strength of the coupling between the two

subsystems, L and R. For $\rho_1 = 0$ and $\rho_2 = 0$ the subsystem L is connected to the subsystem R in only one direction, $L \rightarrow R$; one molecule entering the subsystem R can never return to the L and multiple reflections do not exist. As the fractions $\rho_{1,2}$ increase from $\rho_{1,2} = 0$ up to $\rho_{1,2} = 1$, the occurrence of reflection events increases, and reaches a maximum value and then decreases again to zero.

The analysis of the kinetic equations shows that the system can be operated under stationary conditions provided that the influx rate J is less than a certain critical value, $J_{critical}$

$$J < J_{critical} \qquad (74)$$

The flux $J_{critical}$ represents the carrying capacity of the system. For a stationary process the total transit time is related to the influx rate J by means of Easterby's relationship:

$$\langle t \rangle = (\sum_{u=1}^{N_\Sigma} I_u)/J \qquad (75)$$

where $\sum_{u=1}^{N_\Sigma} I_u$ is the sum of the pool concentrations of chemical intermediates in the system. From eqs 72 and 73 it follows that for a stationary state to exist it is necessary that the total transit time is bigger than a critical value, $\langle t \rangle_{critical}$, which depends on the critical flux $J_{critical}$:

$$\langle t \rangle > \langle t \rangle_{critical} = (\sum_{u=1}^{N_\Sigma} I_u)/J_{critical} \qquad (76)$$

In order to avoid the numerical difficulties generated by the handling of a large number of rate coefficients we assume that all rate coefficients corresponding to the chemical reactions 57−59 and 62−66 are equal to unity. Concerning the reactions 60 and 61, which make the connection between the two subsystems, we assume that they are characterized by total rates, $k_L^{(1)} + k_R^{(1)}$ and $k_L^{(2)} + k_R^{(2)}$ equal to unity, and assume that both fractions, ρ_1 and ρ_2, are equal to each other $\rho = \rho_1 = \rho_2 = $ constant. In the following we use the fraction $\rho = \rho_1 = \rho_2$ as a tuning parameter for generating different reflection regimes in the system. The influx rate, J, and the total concentrations of the four enzymes present in the system, E_L^1, E_L^2, E_R^1, and E_R^2, are chosen in such a way that the restrictions (74)−(76) are fulfilled and the system displays a stable stationary state. For a given numerical computation the value of the influx rate and of the total concentrations of the four enzymes were kept constant. We have run different sets of numerical computations by varying the values of these five parameters and the corresponding results have the same qualitative behavior.

An analysis of the evolution equations of asymmetric systems developed in section 5 shows that a key factor in determining the dynamics of the process is the probability of a reflection pair:

$$\lambda = \alpha_L \alpha_R \qquad (77)$$

Therefore, in our numerical computations we have studied the dependence of the different variables characterizing the system as a function of λ, rather than as a function of the fraction $\rho = \rho_1 = \rho_2$. We have solved the secular equation for the whole system described by the chemical reactions 57−66 and evaluated the probabilities $\psi_e^{(L)}(\theta) \, d\theta$, $\psi_e^{(R)}(\theta) \, d\theta$, $\psi_s^{(L)}(\theta) \, d\theta$, and $\psi_s^{(R)}(\theta) \, d\theta$ in terms of these eigenvalues. With the help of eqs 47, 71, and 77, we have determined the dependence of the crossing probability $T_2 = T_2(\lambda)$ as a function of the probability of
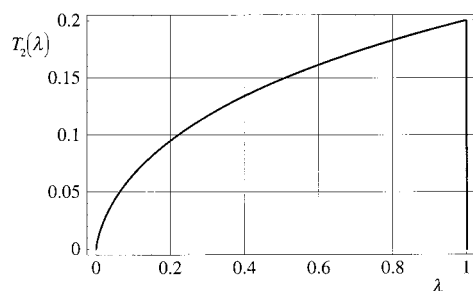
Figure 3. Graphical representation of the dependence the total crossing probability (probability of net transformation), $T_2(\lambda)$, as a function of the probability λ of the occurrence of a reflection pair. Due to the effect of multiple reflections, the probability of net transformation $T_2(\lambda)$ increases with the increase of the probability λ and as $\lambda \rightarrow 1$ it tends toward a limit value close to 0.2. For $\lambda = 1$, however, the net probability of transformation $T_2(\lambda)$ drops to zero and the chemical transformation does not occur.
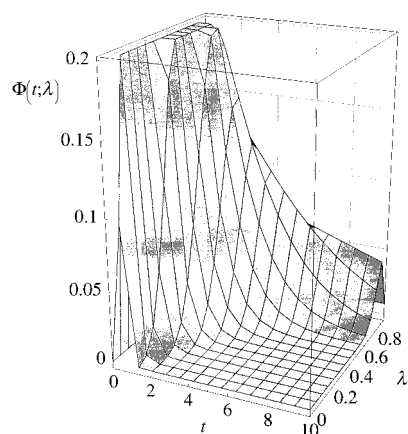

Figure 4. Surface representation of the dependence of the normalized probability density of the crossing (transformation) time, $\Phi(t) = \Phi(t;\lambda)$, on the transformation time t and on the probability λ of occurrence of a pair of reflection events. The probability density becomes broader as the probability λ increases.

occurrence of a pair of reflection events. This dependence is displayed in Figure 3. The function $T_2(\lambda)$ increases monotonically from the initial value $T_2(\lambda=0) = 0$ and as $\lambda \rightarrow 1$ tends toward a limit value close to 0.2. For the limit case where the probability of a reflection pair has the maximum value $\lambda = 1$ the probability of crossing the system falls off to zero, $T_2(\lambda=1) = 0$. These results are consistent with the theoretical model suggested in this article: as λ increases, the probability of crossing the system increases due to an increased contribution of the multiple reflections. In the limit case $\lambda = 1$, a particle moves forward and backward between the two subsystems forever and never leaves the system and therefore $T_2(\lambda=1) = 0$. As suggested by our theory the process of amplification of the probability of net transformation due to multiple reflections occurs at the expense of slowing down the process of chemical transformation. In order to illustrate this effect we have computed the normalized probability density of the crossing (transformation) time, $\Phi(t) = \Phi(t;\lambda)$ as a function of the transformation (crossing) time t and of the probability λ of occurrence of a pair of reflection events. A surface representation of this dependence is given in Figure 4. We notice that the numerical results are consistent with our theoretical predictions. As the probability λ increases the number of reflection events within the system increase and due to this the transformation (crossing) time tends to increase. This effect is clearly displayed by Figure 4, which shows that as λ increases the probability density $\Phi(t) = \Phi(t;\lambda)$ becomes broader and broader. Figures 5
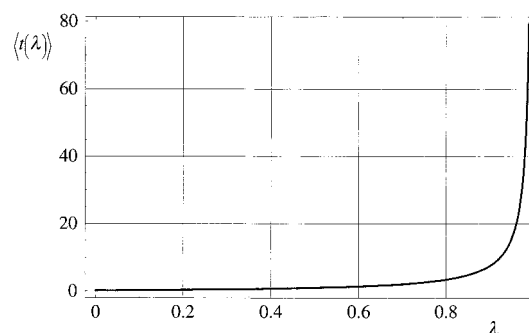

Figure 5. Plot of the average value of the transformation (crossing) time, $\langle t(\lambda) \rangle$, versus the probability λ of occurrence of a pair of reflection events. The average transformation time $\langle t(\lambda) \rangle$ diverges to infinity in the limit $\lambda \rightarrow 1$.
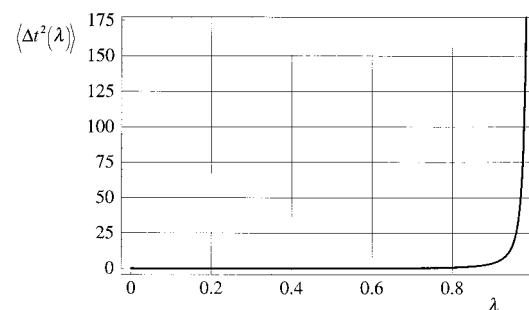

Figure 6. Plot of the dispersion of the transformation (crossing) time, $\langle \Delta t^2(\lambda) \rangle$, versus the probability λ of occurrence of a pair of reflection events. The average transformation time $\langle \Delta t^2(\lambda) \rangle$ diverges to infinity in the limit $\lambda \rightarrow 1$.

and 6 display the dependence of the average value, $\langle t(\lambda) \rangle$, and the dispersion, $\langle \Delta t^2(\lambda) \rangle$, of the crossing (transformation) time t as functions of the probability λ of the occurrence of a number of reflection pairs. We notice that as $\lambda \rightarrow 1$ both the average value $\langle t(\lambda) \rangle$ and the dispersion $\langle \Delta t^2(\lambda) \rangle$ diverge to infinity, a result which is consistent with our prediction that in the limit $\lambda \rightarrow 1$ there is no net transformation.

In conclusion, the numerical calculations presented in this section confirm the main results of our theory. The total probability of crossing T_2 increases with the increase of the probability of occurrence of a number of reflection pairs and tends toward a maximum limit value. However, if the probability of a reflection pair is equal to 1, the total probability of net crossing collapses to zero. On the other hand, the duration of the process increases with the increase of the number of reflection events.

7. Analogy with the Thermally Activated Passage over a Succession of Energy Barriers

In this section we return to the study of symmetric systems and present a simple example which shows that the amplification process due to multiple reflections may occur in the case of other physical and chemical systems.

We consider a symmetric activation energy profile of the type presented in Figure 7. A central symmetric barrier, labeled C, is surrounded by two lateral barriers labeled L (to the left of the central barrier) and R (to the right of the central barrier). The symmetry axis of the central barrier is at the same time the symmetry axis of the whole system and is chosen to be the coordinate axis. The lateral barriers have the same height and shape but they are nor necessarily identical with the central barrier.

A suitable approach for studying the passage over the barriers depicted in Figure 7 is the use of a Fokker−Planck equation
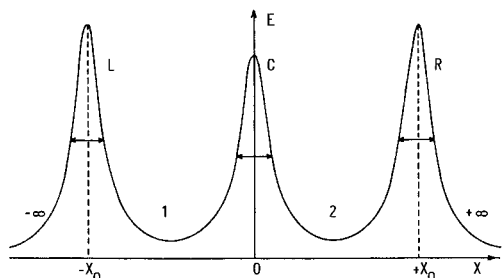
Transit Time Distribution for Biochemical Networks

J. Phys. Chem. B, Vol. 103, No. 19, 1999 **3973**

Figure 7. Graphical representation of a symmetric system of three energy barriers generating the amplification of the escape process due to multiple reflections. The potential energy E is represented as a function of a scalar reaction coordinate X.

for the joint probability density $P(X,Q;t)$ of the reaction coordinate X and of the conjugate moment Q for solving the corresponding Kramers problem.[23] This is a very complicated problem and we do not intend to solve it here. For our purposes it is enough to use a simplified description similar to the one used for the study of resonant activation.[24] Instead of describing the position of a moving particle in terms of the reaction coordinate X, we divide the X axis into four different regions and use an overall probabilistic description, which is given by the probabilities of occurrence of the particle in each of the four regions. We denote by $-\infty$ the region with the reaction coordinate between $-\infty$ and $-X_0$; 1 the region with the reaction coordinate between $-X_0$ and 0; 2 the region with the reaction coordinate between 0 and $+X_0$; and $+\infty$ the region with the reaction coordinate between $+X_0$ and $+\infty$. Here $\pm X_0$ are the reaction coordinates corresponding to the tops of the lateral barriers, respectively. The states 1 and 2 taken together make up the system and the states $\pm\infty$ make up the environment.

The analogy between the biochemical network presented in Figure 1 and the system of energy barrier introduced in this section is obvious. To emphasize this analogy we have used the same notations in both cases. The states $\pm\infty$ in Figure 7 correspond to the environment of the biochemical network in Figure 1 whereas the two subnetworks in Figure 1 correspond to the states 1 and 2 in Figure 7.

We consider a statistical ensemble of independent particles initially placed either in the state 1 or 2 in Figure 7. In the long run all these particles cross some of the energy barriers and escape to the environment, either to $-\infty$ or to $+\infty$. This process of escape can be described by the evolution equations for symmetrical biochemical systems studied in sections 2, 3, and 4. In particular, by repeating the analysis we arrive at eq 16 for the probability densities $\Pi_{L;R}(t)$ of the crossing time of a particle from the system to the environment by crossing the left (L) or the right (R) barrier, respectively. Further, from this expression we can recover eq 20 for the probability T_2 that a particle leaves the system by crossing a succession of two barriers, where now the factors α and β, defined by eqs 21 and 22, have the meaning of the probability of individual crossing of crossing the central or of a lateral barrier, respectively. In the case of the present problem, the ratio of these two probabilities, $\epsilon = \alpha/\beta$, defined by eq 21 is correlated by the ratio

$$\epsilon_h = h_{L;R}/h_C \qquad (78)$$

between the height $h_{L;R}$ of the lateral barriers and the height h_C of the central barrier. If the lateral barriers are high and the central barrier is low, $h_C \ll h_{L;R}$, then both ratios are much larger than 1, $\epsilon \gg 1$ and $\epsilon_h \gg 1$ and most crossings occur across the central barrier and $\alpha/\beta \gg 1$. In the other extreme, for $h_C \gg h_{L;R}$ we have $\epsilon \ll 1$, $\epsilon_h \ll 1$, and $\alpha/\beta \ll 1$ and most crossings

take place over the lateral barriers. By examining eq 20 for the transparency factor T_2 of a succession of two energy barriers we notice the existence of the same paradox as in the case of the biochemical system studied in sections 2−4. The total probability of crossing T_2 increases with the increase of the ratios $\epsilon = \alpha/\beta$, and $\epsilon_h = h_{L;R}/h_C$; that is, it increases with the increase of the height $h_{L;R}$ of the lateral barrier and with the decrease of the height of the central barrier h_C. This apparent paradox can be explained in the same way as in the case of the biochemical system studied before: tall lateral barriers and a small central barrier generate a large number of multiple reflections. Even though in this case the individual probability of leaving the system by crossing a succession of two barriers is smaller than in the case of a system with small lateral barriers, by adding the contributions of multiple reflections to the crossing process we end up with a bigger probability T_2.

The analogy between a biochemical network and a succession of activation energy barriers may sound superficial, but establishes the basic mechanism for the amplification of the crossing factors due to multiple reflections. However, for potential energy barriers a detailed study of this phenomenon must be based on the use of Kramers' theory. Work on this problem is in progress and the main results will be presented in another paper.

8. Conclusions

In this paper we have investigated the phenomenon of amplification of the crossing probability of a complex biochemical system due to multiple reflections. The analysis shows that the increase of the residence time of a test particle in a given region of the system leads to an increase of the probability of escaping from the system, a result which apparently contradicts common sense. The physical analysis of the process has shown that this apparent paradox has a simple explanation: it is due to the multiple reflections within the system. The escape process can take place after an arbitrary number of reflections within the system. The total probability of escaping the system can be computed by adding up the contributions of multiple reflections: as a result, if the number of reflections in the system increases, the probability of escaping the system also increases.

A numerical study of this effect has been done for a simple biochemical system. The numerical analysis confirms our theoretical predictions. In biochemical terms the increase of the crossing probability due to multiple reflections can be viewed as the increase of the probability of net transformation due to an increase of the residence time of the reacting species in the system. By controlling the residence time of a given species in the system it is possible to control the efficiency of the transformation process of the species into a desired product. In most biochemical systems the residence times cannot exceed certain biological threshold values and therefore the optimum efficiency of a process corresponds to the maximum reasonable value of the residence time compatible with the biological constraints which the system has to fulfill. The intermediary metabolism is full of enzymatic transformations catalyzed by two or more isoenzymes. For example, in the glycolytic pathway almost every reaction step is catalyzed by at least two isoenzymes. The results presented in this paper show the important role of isoenzymatic chain transformations in increasing the crossing probability, i.e., the probability of transformation of a given metabolite into some other one through a series of enzymatic reactions. When two alternative transformation pathways, one with isoenzymes and the other without isoenzymes, are compared, the former presents a much larger possibility of optimization of the crossing probability. One can

3974 *J. Phys. Chem. B, Vol. 103, No. 19, 1999*

Vlad et al.

conjecture that this property described here could be one of the reasons behind the massive development of isoenzymes in the cell metabolism.

We have also shown that the amplification of the crossing probability due to multiple reflections can occur in the case of a succession of energy barriers. A qualitative theory of the process has been developed by analogy with the random walk approach used for the study of biochemical networks. For energy barriers a more detailed analysis of the process must be based on the use of Kramers' theory.

Although apparently our theory deals with a special class of biochemical systems the mathematical techniques developed in this article have general validity and can be used for the study of other biochemical processes. Of special interest is the technique developed in Section 6 for the computation of the stochastic properties of the transition time for a subnetwork of a large biochemical network. This technique may serve as a starting point for the development of a lumping approach for the study of large networks. Our approach may serve a similar role to the Thevenin or Norton theorems in the theory of electrical circuits. Work on this problem is in progress and will be reported elsewhere.

After submitting this article for publication we have learnt about a recent review[25] of the double-well model for ion-molecule reactions. This article also considers the possibility of multiple reflections within the system and discusses their influence on the reaction efficiency. Since the model discussed in ref 25 is different from the one presented in this paper, it is not possible to make a direct comparison of the results. However, the comparative analysis of the two approaches outlines that there are two different definitions for the transit time distributions: (1) a "census" distribution which refers to a statistical ensemble of particles at a given moment in time and (2) a "cohort" distribution which refers to the statistics of the different transit times of a group of molecules which enter the system at the same time. This distinction between census and cohort distributions is similar to the distinction between the Eulerian and Lagrangian descriptions of motion in fluid mechanics.[26] The differences between these two different types of distributions are well documented in the literature of mathematical demography.[27] In our future research we intend to extend this analysis from demography to chemical kinetics.

Appendix

We differentiate eq 5 term by term with respect to θ, resulting in

$$\frac{\partial}{\partial \theta} l(\theta) = -\psi_e(\theta) - \psi_s(\theta) \tag{A1}$$

We eliminate the functions $\psi_e(\theta)$ and $\psi_s(\theta)$ from eqs 6 and A1 The result of this operation is a differential equation for the survival function $l(\theta)$:

$$\frac{\partial}{\partial \theta} l(\theta) = -l(\theta)[g_e(\theta) + g_s(\theta)] \tag{A2}$$

We integrate this equation with the initial condition $l(t=0) = 1$. We come to

$$l(\theta) = \exp[-\int_0^\theta g_e(\theta')\, d\theta' - \int_0^\theta g_s(\theta')\, d\theta'] \tag{A3}$$

By inserting eq A3 into eq 6 we get eq 7.

References and Notes

(1) Guderian, A.; Dechert, G.; Zeyer, K. P.; Schneider, F. W. *J. Phys. Chem.* **1996**, *100*, 4437.

(2) Förster, A.; Schneider, W. F. *J. Phys. Chem.* **1996**, *100*, 4442.

(3) Hofmann, W.; Müller, J.; Schneider, W. F. *J. Phys. Chem.* **1996**, *100*, 5388.

(4) Dykman, M. I.; Horita, T.; Ross, J. *J. Chem. Phys.* **1995**, *103*, 966.

(5) Sides, S. W.; Rikvold, P. A.; Novotny, M. A. *Phys. Rev.* **1998**, *E57*, 6512.

(6) Doering, C. R.; Gadoua, J. *Phys. Rev. Lett.* **1992**, *69*, 2318.

(7) Zürcher, U.; Doering, C. R. *Phys. Rev.* **1993**, *E47*, 3862.

(8) Rattay, K. M.; McKane, A. J. *J. Phys. A, Math. Gen (U.K.)* **1991**, *24*, 1215.

(9) Dykman, M. I.; Luchinski, D. G.; Manella, R.; McClintock, P. V. E.; Shart, H. E.; Stein, N. D.; Stocks, N. G. *Phys. Lett.* **1994**, *A193*, 61.

(10) Fulinski, A. *Phys. Lett.* **1994**, *A193*, 267.

(11) Hjelmfelt, A.; Schneider, F. W.; Ross, J. *Science* **1993**, *260*, 335.

(12) Hjelmfelt, A.; Ross, J. *J. Phys. Chem.* **1993**, *97*, 7988.

(13) Hjelmfelt, A.; Ross, J. *PNAS* **1994**, *91*:63.

(14) Laplante, J. P.; Payer, M.; Hjelmfelt, A.; Ross, J. *J. Phys. Chem.* **1995**, *99*, 10063.

(15) Lebender, D.; Schneider, F. W. *J. Phys. Chem.* **1994**, *98*, 7533.

(16) Zeyer, K. P.; Dechert, G.; Hohmann, W.; Blittersdorf, R.; Schneider, F. W. *Z. Naturforsch.* **1994**, *49a*, 953.

(17) Lebender, D.; Müller, J.; Schneider, F. W. *J. Phys. Chem.* **1995**, *99*, 4992.

(18) Blittersdorf, R.; Müller, J.; Schneider, F. W. *J. Chem. Educ.* **1995**, *72*, 760.

(19) Moran, F.; Vlad, M. O.; Ross, J. *J. Phys. Chem.* **1997**, *101*, 9410.

(20) Vlad, M. O.; Moran, F.; Ross, J. *J. Phys. Chem.* **1998**, *102*, 4598.

(21) Vlad, M. O.; Pop, A. *Physica* **1989**, *A155*, 276, 311; *Z. Phys. B.* **1989**, *75*, 413.

(22) Weiss, G. H.; Rubin, R. J. *Adv. Chem. Phys.* **1983**, *52*, 363. Haus, J. W.; Kehr, K. W. *Phys. Rep.* **1987**, *150*, 263. Bouchaud, J. P.; Georges, A. *Phys. Rep.* **1990**, *195*, 127.

(23) Kramers, H. A. *Physica* **1940**, *7*, 284. Hänggi, P.; Talkner, P.; Borkovec, M. *Rev. Mod. Phys.* **1990**, *62*, 251.

(24) Van den Broeck, C. *Phys. Rev.* **1993**, *E47*, 4579.

(25) Moylan, C. R.; Brauman, J. I. *Advances in Classical Trajectory Methods*; JAI Press Inc: New York, 1994; Vol. 2, pp 95−114.

(26) Monin, A. S. *Statistical Fluid Mechanics;* MIT Press: Cambridge. MA, 1971; Vol. 1.

(27) Keyfitz, N. *Applied Mathematical Demography*; Wiley: New York, 1977.