Threshold activation for stochastic chemical reactions in microdomains

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The mean time to reach a threshold (MTT) is the mean first passage time for the number of bound molecules to reach a given value. In the theory of chemical reactions involving a small number of ligands and molecules, the MTT represents the first time a given number of binding sites is formed. In that context, the MTT can be used to characterize the stability of chemical processes, especially when they underlie a biological function. Using a Markov-chain description, we compute here the MTT, in terms of fundamental parameters, such as the number of molecules, the ligands and the forward and backward binding rates. We find that the MTT depends non-linearly on the threshold T, and this result may have several applications, ranging from cellular biology to synaptic plasticity. We confirm our analytical computations with Brownian simulations.

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I. INTRODUCTION

Molecular activation occurring in cellular microdomains depends on the binding-unbinding of ligands to specific targets [1,2]. This is for example the case at synapses, where synaptic plasticity, a long lasting process underlying learning and memory [3] can be induced when the concentration of calcium reaches a certain threshold [4]. Another example concerns the cellular response to a double strand DNA (dsDNA) break: the cell can "sense" the number of breaks and may decide to undergo apoptosis or not. Interestingly, a single dsDNA break can be detected and this event is sufficient to activate a global cellular response. The concept of reaching a threshold as the starting point of a cellular response is ubiquitous in biology. We shall mention two other fundamental examples: in the patterning process, occurring in the embryo development, cell differentiation is controlled by a gradient concentration of morphogens and interestingly, the cell fate can change by a small difference in that concentration (the concentration of the decapentaplegic gene (DPP) in insects can activate different genes at different thresholds [5]. Another example concerns the first step of cellular division, where chromosomes need to be attached before being separated [6,7]

We present here several scenarios where an ensemble of particles (molecules, proteins, ions, ligands) interacts with immobile targets via binding and unbinding. We compute the mean first time MTT that the number of bound targets reaches a threshold value T, which is a key event to induce a cellular response. However, because studying the MTT involves discreet events, we cannot use the standard equationsof mass-action law describing chemical reactions. Recently several efforts were made [8,9] to model chemical reactions in small domains. In [10], using a Markov chain description, we developed a theory of chemical reactions in microdomains based on the dynamis of few Brownian particles. Although we obtained estimates for the number and variance of bound molecules, we did not consider the mean time to reach a threshold, which we are now studying. We consider M Brownian molecules inside a microdomain that can bind to immobile targets S modeled generically as

$$M + S \underset{k_{-1}}{\overset{\lambda}{\rightleftharpoons}} MS,$$

where λ is the forward rate at which a M-molecule encounters one of the free targets and k_{-1} is the backward binding rate at which $M-S$ molecules dissociate. The MTT $\overline{\tau_T}$ for the amount of MS molecules at time t, to reach a threshold T is the expectation of the random time

$$\tau_T = \inf\{t > 0 | |MS|(t) = T\}, \tag{1}$$

where $|MS|(t)$ is the number of MS molecules at time t. We estimate here $\overline{\tau_T}$ in several cases: for an ensemble of the targets initially free and distributed on the surface of a closed microdomain, we compute the MTT analytically when the backward rate vanishes ($k_{-1}=0$) and later on when $k_{-1}>0$. We further confirm these formulas with Brownian simulations. Our main results are summarized by formulas (17)–(20), (22), and (23) which extend previous work on the first passage time for discreet process (ch. XII, p. 292 [11] or on diffusion controlled readtions p. 272 [12]. We shall further extend our analysis to diffusing molecules that can disappear (with a Poissonian rate) before binding the target sites, modeled as

$$M + S \overset{\lambda}{\rightarrow} MS, \tag{2}$$

$$M \overset{\mu}{\rightarrow} \varnothing . \tag{3}$$

For that case, we introduce a two-dimensional Markov chain and by counting the number of paths in a Markov graph, we obtain a general expression (40) for the probability and the meant time to reach the threshold T (49). When the number of binding molecules is large compared to the threshold T, we obtain some asymptotic expressions, confirmed by Brownian simulations. Finally, when there are two competitive molecular pathways, we shall evaluate the probability and the MTT for one of them to be activated before the other.

We conclude with some applications to the spindle checkpoint, an event that precedes the mitotic phase in cell division.

II. MTT FOR STOCHASTIC CHEMICAL REACTIONS

We now use a Markov chain to compute the mean first passage time for the number of bound molecules to reach the threshold T. The substrate S and the diffusing molecules M interact as described by equation I. To derive a markov equation for the probability density functions $p_k(t)=\text{Prob}\{|MS|(t)=k||MS|(0)=0\}$ that the number of bound molecules $|MS|$ at time t is equal to k, we study between time t and $t+\Delta t$, the transitions to the k state, coming from states $k-1$, k, and $k+1$ with transition rates $\lambda_{k-1},-(\lambda_k+k_{-1}k),k_{-1}(k+1)$ [10]. p_k (for $0\le k\le S_0$) satisfy

$$\dot{p}_0 = -\lambda_0 p_0 + k_{-1}p_1,$$

$$\dot{p}_k = -(\lambda_k + k_{-1}k)p_k + \lambda_{k-1}p_{k-1} + k_{-1}(k+1)p_{k+1} \quad \text{for} \quad 0 < k < S_0,$$

$$\dot{p}_{S_0} = -(\lambda_{S_0} + k_{-1}S_0)p_{S_0} + \lambda_{S_0-1}p_{S_0-1}, \tag{4}$$

where

$$\lambda_k = \lambda(S_0 - k)(M_0 - k), \tag{5}$$

which is the rate for one of the M_0-k free molecules to reach the S_0-k free binding sites. λ is the binding rate for one molecule M to a single target, it is the reciprocal of the mean first passage time $\bar{\tau}$ for a particle to a target. When the target is small enough, $\bar{\tau}$ can be approximated by the small hole formulas [13–16]. To estimate the mean first time that the number of bound molecules reaches the threshold T, we impose in Eq. (4) that the state $|MS|=T$ is absorbing, which leads to the modified system:

$$\dot{p}_0 = -\lambda_0 p_0 + k_{-1}p_1,$$

$$\dot{p}_k = -(\lambda_k + k_{-1}k)p_k + \lambda_{k-1}p_{k-1} + k_{-1}(k+1)p_{k+1} \quad \text{for} \quad 0 < k < T-1,$$

$$\dot{p}_{T-1} = -[\lambda_{T-1} + k_{-1}(T-1)]p_{T-1} + \lambda_{T-2}p_{T-2},$$

$$\dot{p}_T = \lambda_{T-1}p_{T-1}. \tag{6}$$

where $p_k(t)=\text{Prob}\{|MS(t)|=k\}$ with $0\le k\le T$. By definition

$$p_T(t) = \text{Prob}\{\tau_T \le t\}, \tag{7}$$

where τ_T is the first hitting time to the threshold T,

$$\tau_T = \inf\{t, |MS|(t) = T\}. \tag{8}$$

The MTT $\overline{\tau_T}$ is given by

$$\overline{\tau}_T = \int_0^{+\infty} \text{Prob}\{\tau_T > t\}dt \tag{9}$$

$$= \int_0^{+\infty} [1 - p_T(t)]dt. \tag{10}$$

Equivalently using the normalization condition

$$\sum_0^T p_k(t) = 1, \tag{11}$$

we have the general expression

$$\bar{\tau}_T = \sum_0^{T-1} a_k, \tag{12}$$

where $a_k=\int_0^{+\infty}p_k(t)dt$. To obtain an analytical expression for $\bar{\tau}_T$, we integrate Eq. (6) between 0 and $+\infty$ with the initial conditions $p_0=1,p_k=0$. Using that $p_T(+\infty)=1$ and $p_k(+\infty)=0$ for $k<T$, we get

$$-1 = -\lambda_0 a_0 + k_{-1}a_1,$$

$$0 = -(\lambda_k + k_{-1}k)a_k + \lambda_{k-1}a_{k-1} + k_{-1}(k+1)a_{k+1} \quad \text{for} \quad 0 < k < T-1,$$

$$0 = -[\lambda_{T-1} + k_{-1}(T-1)]a_{T-1} + \lambda_{T-2}a_{T-2},$$

$$1 = \lambda_{T-1}a_{T-1}. \tag{13}$$

equivalently

$$a_{T-1} = \frac{1}{\lambda_{T-1}}, \tag{14}$$

$$a_k = \frac{1}{\lambda_k} + (k+1)k_{-1}\frac{a_{k+1}}{\lambda_k} \quad \text{for} \quad 0 \le k \le T-2. \tag{15}$$

When the binding is irreversible ($k_{-1}=0$), the MTT $\bar{\tau}_T$ is the sum of the forward rates:

$$\tau_T^{irrev} = \frac{1}{\lambda_0} + \frac{1}{\lambda_1} + \ldots + \frac{1}{\lambda_{T-1}}, \tag{16}$$

$$= \frac{1}{\lambda}\sum_{k=0}^{T-1}\frac{1}{(M_0-k)(S_0-k)}. \tag{17}$$

In particular, when $M_0=S_0$ and $M_0\gg 1$, the asymptotic formula for Eq. (17) becomes

$$\tau_T^{irrev} \approx \frac{T}{\lambda M_0(M_0-T)}. \tag{18}$$

In addition, when the diffusing molecules largely exceed the number of targets ($M_0\gg S_0,T$), we further obtain from Eq. (17), the asymptotic formulas,

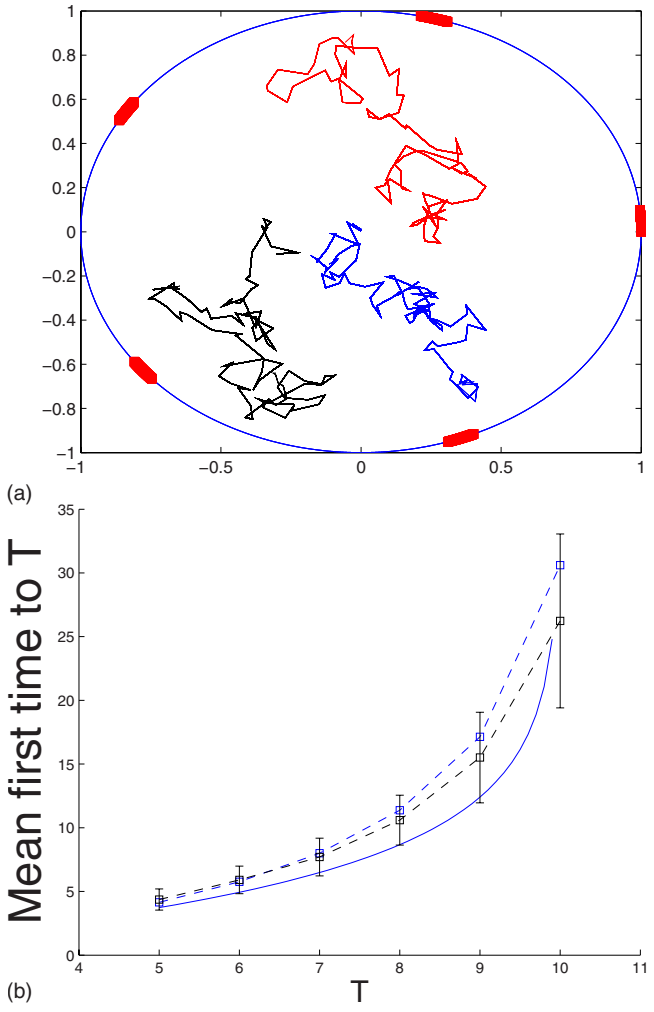


FIG. 1. (Color online) MTT: Up, we present the trajectories of diffusing molecules in a microdomain containing five binding sites on the boundary. Down: we plot the time τ_T^{irrev} as a function of the threshold T. We present the Brownian simulations (dash line, variance in black), the theoretical formula (17) (dash-dot line) and its approximation (19) (solid line) for a circular disk in the irreversible case ($k_{-1}=0$). The other parameters are $S_0=15$, $M_0=10$, $\epsilon=0.05$, $D=0.1\ \mu m^2\ s^{-1}$, and the radius of the disk $R=1\ \mu m$ (we run 200 simulations).

$$\tau_T^{irrev} \approx \begin{cases} \dfrac{1}{\lambda M_0}\log\dfrac{S_0}{S_0-T} & \text{when} \quad M_0 \gg S_0, T, \\ \dfrac{1}{\lambda S_0}\log\dfrac{M_0}{M_0-T} & \text{when} \quad S_0 \gg M_0, T, \\ \dfrac{T}{\lambda M_0 S_0} & \text{when} \quad M_0, S_0 \gg T. \end{cases} \tag{19}$$

In Fig. 1, we plot the MTT τ_T^{irrev} for several values of the threshold T and we compare it with Brownian simulations performed in a circular disk $\Omega=D(R)$, which boundary is reflecting except at the targets.

When $k_{-1}>0$, the analytical expression for $\bar{\tau}_T$ is given by

$$\begin{aligned} \bar{\tau}_T &= \sum_{k=0}^{T-1}\frac{1}{\lambda_k} + k_{-1}\sum_{k=1}^{T-1}\frac{k}{\lambda_k\lambda_{k-1}} + k_{-1}^2\sum_{k=2}^{T-1}\frac{k(k-1)}{\lambda_k\lambda_{k-1}\lambda_{k-2}} \\ &\quad + k_{-1}^3\sum_{k=3}^{T-1}\frac{k(k-1)(k-2)}{\lambda_k\lambda_{k-1}\lambda_{k-2}\lambda_{k-3}} + \cdots \\ &= \sum_{j=0}^{T-1}\left(k_{-1}^j\sum_{k=j}^{T-1}\frac{k!}{(k-j)!\Pi_{i=k-j}^{k}\lambda_i}\right) \\ &= \frac{1}{\lambda}\sum_{j=0}^{T-1}\left(\frac{k_{-1}}{\lambda}\right)^j\sum_{k=j}^{T-1}\frac{k!}{(k-j)!}\frac{(M_0-k-1)!}{(M_0-k+j)!}\frac{(S_0-k-1)!}{(S_0-k+j)!}. \end{aligned}$$

Finally, we obtain

$$\bar{\tau}_T = \frac{1}{\lambda}\sum_{j=0}^{T-1}\left(\frac{k_{-1}}{\lambda}\right)^j\sum_{k=j}^{T-1}\frac{k!}{(k-j)!}\frac{(M_0-k-1)!}{(M_0-k+j)!}\frac{(S_0-k-1)!}{(S_0-k+j)!}. \tag{20}$$

This sum can be further approximated for the three following regimes $M_0 \gg S_0, T, S_0 \gg M_0, T$, and $S_0=M_0 \gg T$, by using the first-order expansion in $\frac{k_{-1}}{\lambda}$ only and the sum $\Sigma_1^N \frac{1}{k}=\log(N)+O(1)$. We obtain,

$$\bar{\tau}_T \approx \begin{cases} \tau_T^{irrev} + \dfrac{k_{-1}}{(\lambda M_0)^2}\left[\dfrac{T}{S_0-T} - \log\left(1+\dfrac{T}{S_0-T}\right)\right], \\ \text{when} \quad M_0 \gg S_0, T, \\ \tau_T^{irrev} + \dfrac{k_{-1}}{(\lambda S_0)^2}\left[\dfrac{T}{M_0-T} - \log\left(1+\dfrac{T}{M_0-T}\right)\right] \\ \text{when} \quad S_0 \gg M_0, T, \\ \tau_T^{irrev} + \dfrac{k_{-1}}{2\lambda^2}\left(\dfrac{T}{M_0^2}\right)^3, \quad \text{when} \quad S_0 = M_0 \gg T. \end{cases} \tag{21}$$

Using the expression for τ^{irrev} [Eq. (19)], we finally obtain the asymptotic expressions:

$$\bar{\tau} \approx \begin{cases} \dfrac{1}{\lambda M_0}\left\{\log\left(1+\dfrac{T}{S_0-T}\right) + \dfrac{k_{-1}}{\lambda M_0}\left[\dfrac{T}{S_0-T} - \log\left(\dfrac{S_0}{S_0-T}\right)\right]\right\} \\ \text{when} \quad M_0 \gg S_0, T, \\ \dfrac{1}{\lambda S_0}\left\{\log\left(\dfrac{M_0}{M_0-T}\right) + \dfrac{k_{-1}}{\lambda S_0}\left[\dfrac{T}{M_0-T} - \log\left(\dfrac{M_0}{M_0-T}\right)\right]\right\} \\ \text{when} \quad S_0 \gg M_0, T, \\ \dfrac{T}{\lambda M_0^2}\left[1 + \dfrac{k_{-1}}{2\lambda^2}\left(\dfrac{T}{M_0^2}\right)^2\right] \quad \text{when} \quad S_0 = M_0 \gg T. \end{cases} \tag{22}$$

Furthermore, when $T \ll S_0$ and $T \ll M_0$ respectively, in the two first regimes [Eq. (22)], we obtain the refined estimates

$$\bar{\tau} \approx \begin{cases} \dfrac{T}{\lambda M_0 S_0}\left[1+\dfrac{1}{2}\left(1+\dfrac{k_{-1}}{\lambda M_0}\right)\left(\dfrac{T}{S_0}\right)\right], \\ \quad \text{when} \quad M_0 \gg S_0 \gg T, \\ \dfrac{T}{\lambda M_0 S_0}\left[1+\dfrac{1}{2}\left(1+\dfrac{k_{-1}}{\lambda S_0}\right)\left(\dfrac{T}{M_0}\right)\right], \\ \quad \text{when} \quad S_0 \gg M_0 \gg T. \end{cases} \tag{23}$$

We conclude that for $\bar{\tau} \gg 1$, the time to threshold T τ_T varies quadratically with the narrow escape time $\bar{\tau}$ (as $\bar{\tau}=\frac{1}{\lambda}$), however, it is a nonlinear increasing function of T. These computations we presented are quite general and can be applied to describe the mean time to a given number of bound molecules for any chemical reactions. In particular, changing the threshold can be seen as a modulatory mechanism.

III. MTT FOR CHEMICAL REACTIONS IN A DOMAIN WITH KILLING PROCESS

We now study in a microdomain with binding sites, the MTT of diffusing molecules which can also be killed before hitting the sites. In that case, the threshold may never be reached. We shall compute first the probability to the threshold and second the MTT. We treat the case of irreversible binding sites only and model the killing process as Poissonian of parameter μ. To derive the probability to reach the threshold T, we use a two-dimensional Markov chain for the joint probability density function $p_{k,m}(t)$ that at time t, there are k bound molecules and it remains $m-k$ free diffusing molecules.

$$p_{k,m}(t) = P\{|MS|(t) = k, w(t) = m, |MS|(0) = 0, w(0) = M_0\},$$

where $w(t)=|M|(t)+|MS|(t)$. In the irreversible case, the transitions to the state (k,m) between time t and $t+\Delta t$ can only occur from the states $(k-1,m)$, (k,m) and $(k,m+1)$. To compute the probability that the threshold T is achieved, we study the chain (k,m) of having k bound molecules and m-k free remaining molecules. By imposing a boundary condition at (T,m) and $(k,T-1)$, we will obtain the probability that the threshold is reached before the molecules are degraded by summing over the state (T,m) for $m=T \ldots M_0$. We represent the transition diagram between states in Fig. 2 and the master equations are

$$\dot{p}_{0,M_0} = -(\lambda M_0 S_0 + \mu M_0) p_{0,M_0},$$

$$\dot{p}_{0,m} = \mu(m+1)p_{0,m+1} - (\lambda S_0 m + \mu m)p_{0,m} \quad \text{for} \quad T-1 < m < M_0,$$

$$\dot{p}_{k,M_0} = \lambda(S_0-k+1)(M_0-k+1)p_{k-1,M_0} - [\lambda(S_0-k)+\mu](M_0-k)p_{k,M_0} \quad \text{for} \quad 0 < k < T,$$

$$\dot{p}_{T,m} = \lambda(S_0-T+1)(m-T+1)p_{T-1,m} \quad \text{for} \quad T \le m \le M_0,$$

$$\dot{p}_{k,T-1} = \mu(T-k)p_{k,T} \quad \text{for} \quad 0 \le k \le T-1,$$

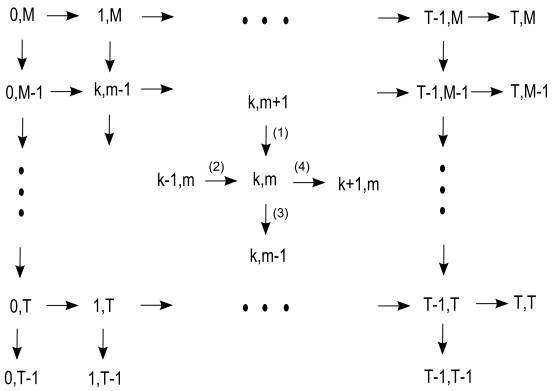


FIG. 2. Diagram of transition between states. Transition rates (1): $\mu(m-k+1)$ (2): $\lambda(S-k+1)(m-k+1)$ (3): $\lambda(S-k)(m-k)$ (4): $\mu(m-k)$ where λ and μ are, respectively, the forward binding rate and the killing rate.

$$\dot{p}_{k,m} = \lambda(S_0-k+1)(m-k+1)p_{k-1,m} + \mu(m+1-k)p_{k,m+1} - [\lambda(S_0-k)+\mu](m-k)p_{k,m} \quad \text{for} \quad T-1 < m < M_0, \quad 0 < k < T, \tag{24}$$

where λ and μ are, respectively, the forward binding rate and the killing rate.

The initial condition reads $p_{0,M_0}(0)=1$ and the normalization condition is

$$\sum_{k=0,m=0}^{M_0,N_0} p_{k,m} = 1. \tag{25}$$

To derive the steady state probabilities $p_{T,m}(\infty)$ and $p_{k,T-1}(\infty)$, we shall now use that the only absorbing states (T,m) and $(k,T-1)$ are given for $T \le m \le M_0$ and $0 \le k \le T-1$ (see Fig. 2). Thus by integrating over time system (24), we obtain that

$$q_{0,M_0} = \frac{1}{M_0(\lambda S_0 + \mu)}, \tag{26}$$

$$q_{0,m} = \frac{1}{m(\lambda S_0+\mu)}\left(\frac{\mu}{\lambda S_0+\mu}\right)^{M_0-m} \quad \text{for} \quad T-1 < m < M_0, \tag{27}$$

$$q_{k,M_0} = \frac{\lambda^k S_0!}{(S_0-k)!(M_0-k)\Pi_{j=0}^{k}[\mu+\lambda(S_0-j)]} \quad \text{for} \quad 0 < k < T, \tag{28}$$

$$p_{T,m}(\infty) = \lambda(S_0-T+1)(m-T+1)q_{T-1,m} \quad \text{for} \quad T \le m \le M_0, \tag{29}$$

$$p_{k,T-1}(\infty) = \mu(T-k)q_{k,T} \quad \text{for} \quad 0 \le k \le T-1, \tag{30}$$

$$q_{k,m} = \frac{[\mu q_{k,m+1} + \lambda(S_0 - k + 1)(m - k + 1)q_{k-1,m}]}{(m-k)[\mu + \lambda(S_0 - k)]} \times (m + 1 - k) \quad \text{for} \quad T - 1 < m < M_0, \quad 0 < k < T, \tag{31}$$

where for $0 \le k \le T-1$, $T \le m \le M_0$,

$$q_{k,m} = \int_0^\infty p_{k,m}(t)dt. \tag{32}$$

A. Probability to reach the threshold

The probability P_T to reach the threshold is equal to the probability to reach any of the states (T,m) for $m=T,..M_0$, that is

$$P_T = \sum_{m=T}^{M_0} p_{T,m}(\infty). \tag{33}$$

To estimate P_T, we study the system of Eqs. (26)–(31) and for $0<k<T$ and $T \le m < M$, we have derived in the Appendix A

$$q_{k,m} = \frac{\left[\prod_{i=0}^{k-1} \frac{\lambda(S_0 - i)}{\lambda(S_0 - i) + \mu}\right] \sum_{0 \le i_1, \ldots, i_{M_0 - m} \le k} \prod_{i_j} \frac{\mu}{\lambda(S_0 - i_j) + \mu}}{[\lambda(S_0 - k) + \mu](m - k)}. \tag{34}$$

We proceed with the computation of the probability P_T to reach the threshold. We get from Eq. (30), for $T \le m < M_0$ that

$$p_{T,m}(\infty) = \lambda(S_0 - T + 1)(m - T + 1)q_{T-1,m}, \tag{35}$$

$$= \left(\prod_{i=0}^{T-1} \frac{\lambda(S_0 - i)}{\lambda(S_0 - i) + \mu}\right) \times \sum_{0 \le i_1, \ldots, i_{M_0 - m} \le T-1} \prod_{i_k} \frac{\mu}{\lambda(S_0 - i_k) + \mu}. \tag{36}$$

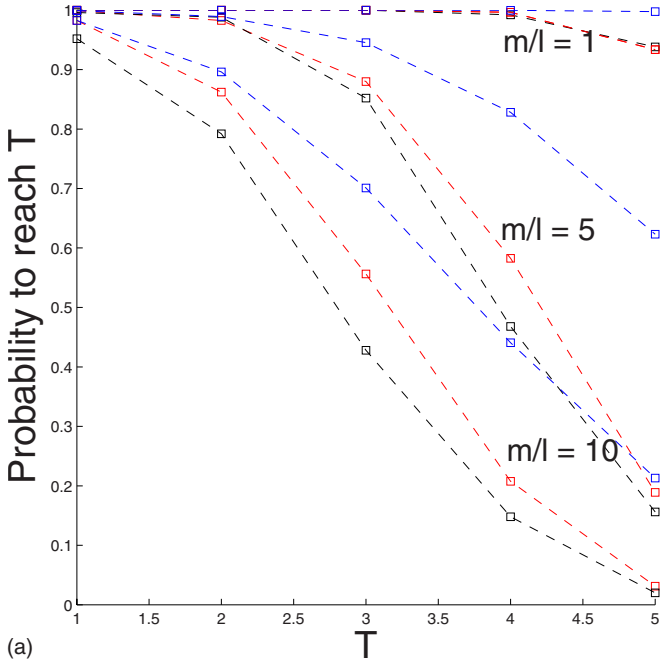
Using Eq. (28),

$$q_{0,m} = \frac{1}{m(\lambda S_0 + \mu)} \left(\frac{\mu}{\lambda S_0 + \mu}\right)^{M_0 - m} \quad \text{for} \quad T - 1 < m < M_0, \tag{37}$$

we also have for $m=M_0$,

$$p_{T,M_0}(\infty) = \left(\prod_{i=0}^{T-1} \frac{\lambda(S - i)}{\lambda(S - i) + \mu}\right). \tag{38}$$

Finally, using that the probability is given by



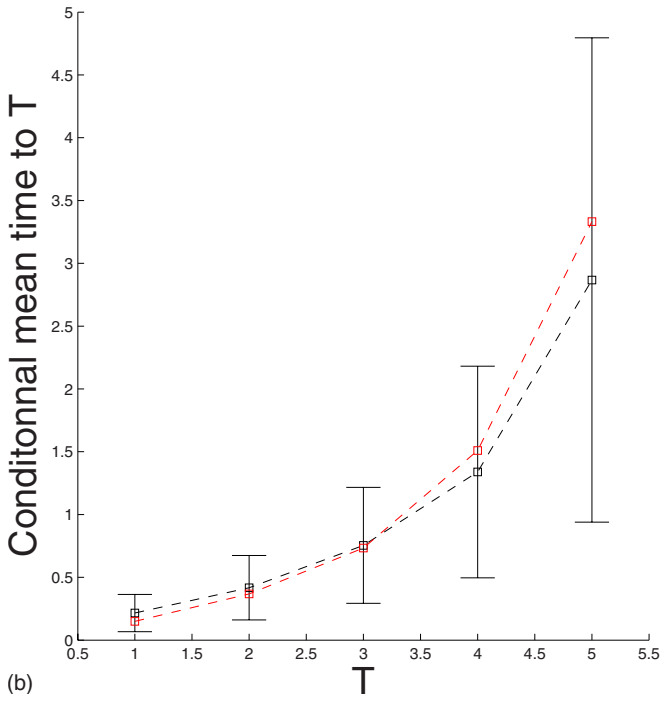


FIG. 3. (Color online) Probability and MTT in a microdomain where ligands can be killed before binding. Up: we plot the probability P_T to reach the threshold as a function of T, for different values of the ratio $\frac{\mu}{\lambda}$ (1,5,10). The exact formula (40) (solid line) is compared with the approximation (43) (dash line). We also compare with Brownian simulations of M molecules (dash-dot line), with diffusion coefficient D moving inside a circular disk of radius R. The binding sites are of size ϵ. Down: we plot the conditional MTT as function of T for $\epsilon=0.05$. The mean first passage time (the initial position is at the center) to a binding site of size ϵ is approximated by $\frac{1}{\lambda} = \tau = (\frac{R^2}{D})[\log(\frac{4\pi}{\epsilon}) + \frac{1}{4}]$ (see [15]). The other parameters are $R=1$ μm, $D=1$ μm s^{-2}, $S=5$, and $M=10$. Number of Brownian simulations=250.

$$P_T = \sum_{m=T}^{M_0} p_{T,m}(\infty), \tag{39}$$

we finally obtain, introducing the variable $\frac{\mu}{\lambda}$ and T,

$$p\left(\frac{\mu}{\lambda},T\right) = P_T = \left(\prod_{i=0}^{T-1} \frac{\lambda(S-i)}{\lambda(S-i)+\mu}\right) \times \left(1 + \sum_{0\le i_1\le T-1} \frac{\mu}{\lambda(S-i_1)+\mu} + \ldots + \sum_{0\le i_1\le ..\le i_{M_0-T}\le T-1} \prod_{i_k} \frac{\mu}{\lambda(S-i_k)+\mu}\right). \tag{40}$$

We show in Fig. 3, the graph of P_T as a function of the threshold T for various values of the ratio $r=\frac{\mu}{\lambda}$. When $\frac{T}{S} \ll 1$, we obtain the approximation

$$p\left(\frac{\mu}{\lambda},T\right) \approx \left(\frac{\lambda S}{\lambda S+\mu}\right)^T \left(1 + \frac{\mu}{\lambda S+\mu}|G_1| + \ldots \left(\frac{\mu}{\lambda S+\mu}\right)^i |G_i| \ldots + \left(\frac{\mu}{\lambda S+\mu}\right)^{M-T} |G_{M_0-T}|\right), \tag{41}$$

where $|G_k|$ is the number of k tuples $(i_1,..i_k)$ where $0\le i_1 \le \ldots \le i_k \le T-1$. Using that $|G_k| = \binom{T-1+k}{k}$, we obtain

$$p\left(\frac{\mu}{\lambda},T\right) \approx \left(\frac{\lambda S}{\lambda S+\mu}\right)^T \sum_{k=0}^{M-T} \binom{T-1+k}{k} \left(\frac{\mu}{\lambda S+\mu}\right)^k, \tag{42}$$

which can written as (see Appendix B)

$$p\left(\frac{\mu}{\lambda},T\right) \approx \sum_{k=T}^{M} \binom{M}{k} \left(\frac{\lambda S}{\lambda S+\mu}\right)^k \left(\frac{\mu}{\lambda S+\mu}\right)^{M-k}. \tag{43}$$

This formula has the following interpretation: when there are many binding sites compared to the number of diffusing molecules, the binding events become independent. Consequently, the probability to bind can be approximated by Bernoulli distribution of parameter $\frac{\lambda S}{\lambda S+\mu}$, and the probability of the binding number is a binomial distribution of parameters $(M, \frac{\lambda S}{\lambda S+\mu})$. Finally, the probability to reach the threshold is equivalent to have at least T bounds, and thus we obtain formula (43). Interestingly, as shown in Fig. 3, already for $r=10$, the exact formula cannot be well approximated by the approximation (41) and the analytical solution should be used. The probability is a decreasing, inverse sigmoid type function as function of the threshold T.

B. Conditional MTT

To get the conditional MTT, we first compute the mean time $\bar{\tau}(\sigma)$ for a trajectory parametrized by $\sigma = (i_0, i_1, i_2, \ldots, i_n)$ where $0=i_0\le i_1 \le \ldots i_{n-1} < i_n = T$ and $1 \le n \le M-T+1$, that follows a path in the Markov diagram (see Fig. 6 in Appendix B).

When there are k bound molecules and it remains $m-k$ free molecules, we shall estimate the mean transition time from this state (k,m), to the state $(k+1,m)$. This event is Poissonian with rate $\lambda_{k,m}=\lambda(S-k)(m-k)$ and the probability of binding before a molecule is killed is given by $\frac{\lambda_{k,m}}{\lambda_{k,m}+\mu(m-k)}$. Thus, the conditional mean binding time is

$$E(\tau^{MS}, |MS| = k, |M| = m-k) = \frac{1}{\lambda_{k,m}} \frac{\lambda_{k,m}}{\lambda_{k,m}+\mu(m-k)} = \frac{1}{\lambda_{k,m}+\mu(m-k)}. \tag{44}$$

Similarly, the mean time to killing is

$$E(\tau^{K}, |MS| = k, |M| = m-k) = \frac{1}{\lambda_{k,m}+\mu(m-k)}. \tag{45}$$

The random times along the path σ are independent, thus the total mean time $\bar{\tau}(\sigma)$ is the sum of all the mean times

$$\bar{\tau}(\sigma) = \begin{cases} \sum_{k=0}^{T-1} \frac{1}{[\lambda(S-k)+\mu](M-k)} & \text{if} \quad \sigma=(0,T) \\ \sum_{j=1}^{n-1} \frac{1}{[\lambda(S-i_j)+\mu](M-j+1-i_j)} + \sum_{j=1}^{n} \sum_{\substack{k=i_{j-1} \\ i_{j-1}\ne i_j}}^{i_j-1} \frac{1}{[\lambda(S-k)+\mu](M-j+1-k)} & \\ \text{otherwise}. & \end{cases}, \tag{46}$$

The probability $P(\sigma)$ that the dynamics follows the path σ is

$$P(\sigma)=\begin{cases}\displaystyle\prod_{i=0}^{T-1}\frac{\lambda(S-i)}{\lambda(S-i)+\mu} & \text{if} \quad \sigma=(0,T)\\ \left(\displaystyle\prod_{i=0}^{T-1}\frac{\lambda(S-i)}{\lambda(S-i)+\mu}\right)\left(\displaystyle\prod_{k=1}^{n-1}\frac{\mu}{\lambda(S-i_k)+\mu}\right) & \\ \text{otherwise}. & \end{cases}. \tag{47}$$

Finally, for a forward binding constant λ, a killing rate μ, the conditional MTT $\bar{\tau}_T(\lambda,\mu,T)$ is

$$\bar{\tau}_T(\lambda,\mu,T)=\sum_{\sigma}\tau(\sigma)P(\sigma|T \text{ is reached}), \tag{48}$$

$$=\frac{\displaystyle\sum_{\sigma}\tau(\sigma)P(\sigma)}{p\left(\frac{\mu}{\lambda},T\right)}, \tag{49}$$

where $p(\frac{\mu}{\lambda},T)$ is the probability to reach the threshold computed in the previous subsection [formula (40)]. We now approximate $\tau_T(\lambda,\mu,T)$ at first order in $\frac{\mu}{\lambda}$, which means that we neglect all the paths $\sigma=(i_0,i_1,i_2,\ldots,i_n)$ such that $n>2$, [the probability for the other paths is at least of order $(\frac{\mu}{\lambda})^2$]. Consequently, considering $\sigma=(0,T)$ and $\sigma=(0,i,T)$ with $0\leq i\leq T-1$. At first order, using formula (41), $p(\frac{\mu}{\lambda},T)=1+o(\frac{\mu}{\lambda})$. In addition, when $M\gg T$, we obtain from relations (46) and (47) the approximations for $0\leq i\leq T-1$

$$P[\sigma=(0,T)]\tau[\sigma=(0,T)] \approx\left[1-\sum_{k=0}^{T-1}\frac{\mu}{\lambda(S-k)}\right]\sum_{k=0}^{T-1}\frac{1}{\lambda M(S-k)}\left[1-\frac{\mu}{\lambda(S-k)}\right],$$

$$P[\sigma=(0,i,T)]\tau[\sigma=(0,i,T)] \approx\left(\frac{\mu}{\lambda}\right)\frac{1}{\lambda M(S-i)}\left(\sum_{k=0}^{T-1}\frac{1}{S-k}+\frac{1}{S-i}\right). \tag{50}$$

Finally, we obtain

$$\tau_T(\lambda,\mu,T)\approx\frac{1}{\lambda M}\log\left(\frac{S}{S-T}\right) \quad \text{when} \quad M\gg T, \quad \lambda\gg\mu. \tag{51}$$

Interestingly, in Eq. (51), the term in $\frac{\mu}{\lambda}$ vanishes, and thus we recover the zero order approximation Eq. (19) for the MTT when particles cannot escape. In Fig. 3, the analytical formula (49) and the result of Brownian simulations show reasonable agreement. It might be tempting to believe that replacing degradation by a small absorbing window would give similar results. It does not. Indeed, the probability to reach a window in a sphere containing several others depends nonlinearly on their distribution, through their capacitance [17–19]. Thus, the rates $\lambda_{k,m}$ and $\mu_{k,m}$ will differ from the ones we obtained here. The geometrical configuration of the holes will now influence the escape and binding rates, and this effect should be studied carefully.

IV. COMPETING THRESHOLDS

When m molecules M can bind to sites S_1 (of number s_1) with a rate k_1 or to sites S_2 (of number s_2) with a rate k_2 (no backward rate),

$$M+S_1\overset{k_1}{\rightarrow}MS_1, \tag{52}$$

$$M+S_2\overset{k_2}{\rightarrow}MS_2, \tag{53}$$

we propose to estimate the probability that the threshold T_1 of MS_1 bindings is reached before that the number of bound MS_2 reaches the threshold T_2. We will also compute the corresponding conditional MTT.

When $T_1+T_2\leq m$ (for $T_1+T_2\geq m$, the thresholds could possibly not be reached), the analysis uses a two-dimensional Markov chain. Adapting Eq. (24), the probability density function $p_{i,j}(t)$ for i bindings MS_1 and j bindings MS_2 ($0\leq i\leq T_1$ and $0\leq j\leq T_2$), satisfies the Markov equation

$$\dot{p}_{0,0}=-(k_1ms_1+k_2ms_2)p_{0,0},$$

$$\dot{p}_{i,0}=-(k_1(s_1-i)+k_2s_2)(m-i)p_{i,0}+k_1(s_1-i+1)(m-i+1)p_{i-1,0},$$

$$\dot{p}_{0,j}=-[k_1s_1+k_2(s_2-j)](m-j)p_{0,j}+k_2(s_2-j+1)(m-j+1)p_{0,j-1},$$

$$\dot{p}_{T_1,j}=k_1(s_1-T_1+1)(m-j-T_1+1)p_{T_1-1,j},$$

$$\dot{p}_{i,T_2}=k_2(s_2-T_2+1)(m-i-T_2+1)p_{i,T_2-1},$$

$$\dot{p}_{i,j}=[k_1(s_1-i)+k_2(s_2-j)](m-i-j)p_{i,j}+(m-i-j+1)\times[k_1(s_1-i+1)p_{i-1,j}+k_2(s_2-j+1)p_{i,j-1}].$$

and the time-integrated equations are

$$-1=-(k_1ms_1+k_2ms_2)q_{0,0},$$

$$0=-[k_1(s_1-i)+k_2s_2](m-i)q_{i,0}+k_1(s_1-i+1)(m-i+1)q_{i-1,0},$$

$$0=-[k_1s_1+k_2(s_2-j)](m-j)q_{0,j}+k_2(s_2-j+1)(m-j+1)q_{0,j-1},$$

$$p_{T_1,j}(\infty)=k_1(s_1-T_1+1)(m-j-T_1+1)q_{T_1-1,j},$$

$$p_{i,T_2}(\infty)=k_2(s_2-T_2+1)(m-i-T_2+1)q_{i,T_2-1},$$

$$0=[k_1(s_1-i)+k_2(s_2-j)](m-i-j)q_{i,j}+(m-i-j+1)\times[k_1(s_1-i+1)q_{i-1,j}+k_2(s_2-j+1)q_{i,j-1}]\,.,$$

If $\tau(T_1)$ (respectively, $\tau(T_2)$) is the first time the threshold T_1

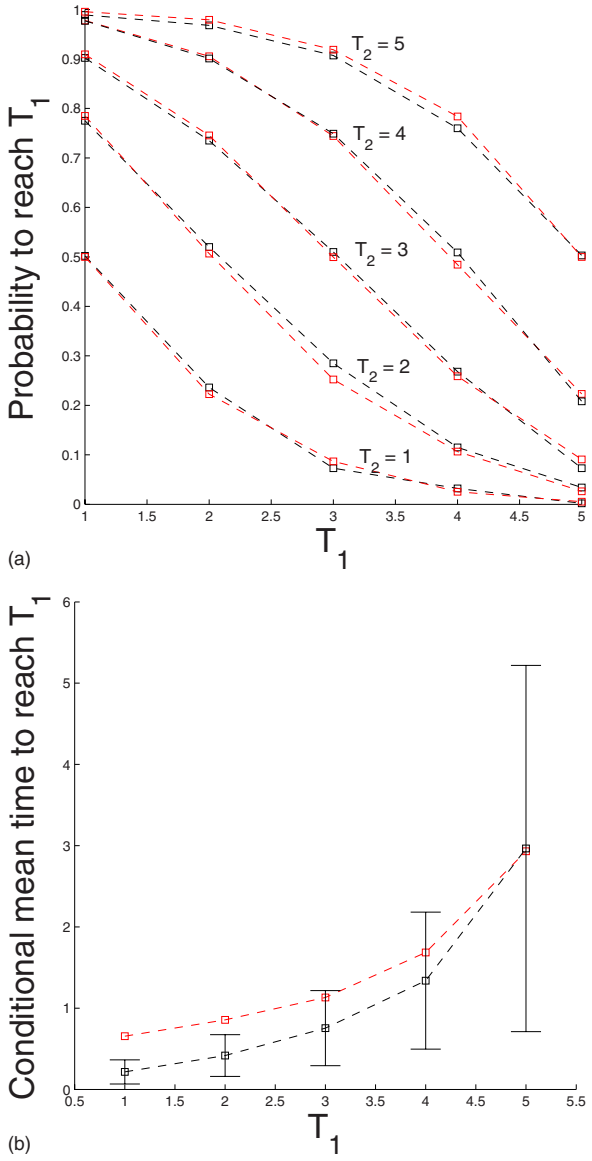


FIG. 4. (Color online) Probability and Mean time to reach the threshold T_1 before T_2 in a microdomain where ligands bind with two kind of sites S_1 and S_2. Up: we plot the probability $P[\tau(T_1) < \tau(T_2)]$ to reach the threshold as a function of T_1 for different values of T_2. We present the exact formula (57) (solid line) and results obtained by Brownian simulations (dash line) in a circular domain of radius R with s_1 targets S_1 of size ϵ_1 and s_2 targets S_2 of size ϵ_2. Down: we plot the conditional MTT as function of T_1 for $T_2=5$. The parameters are $S_1=S_2=5$, $M=10$, $\epsilon_1=\epsilon_2=0.05$ μm, $R=1$ μm, and $D=1$ $\mu m^2\,s^{-1}$. The rates k_1 and k_2 are given by $\frac{1}{\tau}$ where $\tau=\frac{R^2}{D}[\log(\frac{4\pi R}{\epsilon})+\frac{1}{4}]$ (see [15]). We ran 500 simulations.

is reached (respectively, T_2, the probability that T_1 is reached before T_2 is

$$P(\tau(T_1) < \tau(T_2)) = \sum_{j=0}^{T_2-1} p_{T_1,j}(\infty). \tag{54}$$

Using similar combinatorial considerations as in Sec. III A, the probability P_σ of a path $\sigma=(i_0,i_1,\ldots,i_n)$ for $0=i_0\leq i_1 \leq \ldots \leq i_n=T_1$ and $1\leq n\leq T_2$ is

$$P_\sigma = \prod_{k=0}^{n-1} f(i_k,i_{k+1}), \tag{55}$$

where i_1 counts the last bindings between M and S_1, before any previous bindings between M and S_2. In general, i_k is defined such that $i_{k+1}-i_k$ is the number of MS_1 bounds following exactly k-th MS_2 bounds. If $i_1=i_2$, there is another consecutive bound between a single M and another single S_2 molecules, while for $i_1<i_2$, there are i_2-i_1 bindings between M and S_1, followed by a single binding between M and S_2 and so on. For $k<n-1$, $f(i_k,i_{k+1})$ are the transition probabilities between the states $(|MS_1|,|MS_2|)=(i_k,k),(i_{k+1},k+1)$. For the special case $k=n-1$, $f(i_{n-1},T_1)$ is the transition probability between the states $(i_{n-1},n-1),(i_n,n-1)$. The computation of f goes as follow: for $i_k=i_{k+1}$, the transition probability is the one of a M molecule to bind to one of the $s_2-i_kS_2$ free binding sites where there are s_1-k free S_1 sites, that is $\frac{k_2(s_2-i_k)}{k_1(s_1-k)+k_2(s_2-i_k)}$.

$$f(i_k,i_{k+1}) = \begin{cases} \dfrac{1}{1+\dfrac{k_1(s_1-i_k)}{k_2(s_2-k)}} & \text{if } i_k = i_{k+1} \\ \dfrac{1}{1+\dfrac{k_1(s_1-i_{k+1})}{k_2(s_2-k)}} \displaystyle\prod_{j=i_k}^{i_{k+1}-1} \dfrac{1}{1+\dfrac{k_2(s_2-k)}{k_1(s_1-j)}} & \text{if } i_k \neq i_{k+1} \text{ and } k<n-1 \\ \displaystyle\prod_{j=i_k}^{i_{k+1}-1} \dfrac{1}{1+\dfrac{k_2(s_2-k)}{k_1(s_1-j)}} & \text{if } k=n-1, \end{cases} \tag{56}$$

where the third case corresponds to $i_{k+1}-i_kMS_1$ bindings followed by a MS_2 single event. Summing over all possible paths,

$$P[\tau(T_1) < \tau(T_2)] = \sum_{k=0}^{T_2-1} \left[\sum_{\sigma=(0=i_0\leq\ldots\leq i_{k+1=T_1})} P_\sigma \right]. \tag{57}$$

In Fig. 4 we plotted using formula (57) the probability $P[\tau(T_1)<\tau(T_2)]$ as a function of T_1 for fixed T_2. Using the argumentation of formula (43), when $T_1 \ll s_1$ and $T_2 \ll s_2$, this probability can be approximated by

$$P(\tau(T_1) < \tau(T_2)) \approx \sum_{k=T_1}^{T_1+T_2-1} \binom{T_1+T_2-1}{k} \left(\frac{k_1 s_1}{k_1 s_1 + k_2 s_2}\right)^k \times \left(\frac{k_2 s_2}{k_1 s_1 + k_2 s_2}\right)^{T_1+T_2-1-k}. \tag{58}$$

We remark that P depends on the ratio $\frac{k_1}{k_2}$ only, but not on the number m of M molecules provided that $T_1+T_2\leq M$. The conditional MTT to reach the threshold T_1 before T_2 is

$$\tau(k_1,k_2,T_1,T_2) = \frac{\Sigma_\sigma \tau_\sigma P(\sigma)}{P[\tau(T_1) < \tau(T_2)]}, \tag{59}$$

where

$$\tau_\sigma = \sum_{k=0}^{n-1} g(i_k, i_{k+1}), \tag{60}$$

and where

$$g(i_k,i_{k+1}) = \begin{cases} \dfrac{(1/k_2)}{(m-i_k-k)(s_2-k)} & \text{if} \quad i_k = i_{k+1} \\ \displaystyle\sum_{j=i_k}^{i_{k+1}} \dfrac{(1/k_1)}{(m-j-k)(s_1-j-k)} & \\ + \dfrac{(1/k_2)}{(m-i_{k+1}-k)(s_2-k)} & \text{if} \quad i_k \neq i_{k+1} \\ & \text{and} \quad k < n-1 \\ \displaystyle\sum_{j=i_k}^{i_{k+1}-1} \dfrac{(1/k_1)}{(m-j-k)(s_1-j-k)} & \text{if} \quad k = n-1. \end{cases}$$

Following the computation of Sec III B, for $\frac{k_1}{k_2}, m \gg 1$, we approximate the conditional MTT $\bar{\tau}(T_1,T_2)$, as [see Eq. (50)]

$$\begin{aligned} &P[\sigma=(0,T_1)]\bar{\tau}[\sigma=(0,T_1)] \\ &\quad \approx \sum_{i=0}^{T_1-1} \frac{1}{k_1 m(s_1-i)} - \frac{k_2}{k_1}\frac{s_2}{k_1 m} \\ &\qquad \times \left[\sum_{i=0}^{T_1-1} \frac{1}{(s_1-i)^2} + \left(\sum_{i=0}^{T_1-1} \frac{1}{s_1-i} \right)^2 \right], \end{aligned}$$

$$\begin{aligned} &P[\sigma=(0,j,T_1)]\bar{\tau}[\sigma=(0,j,T_1)] \\ &\quad \approx \frac{k_2}{k_1}\frac{s_2}{m(s_1-j)} \times \left[\frac{1}{s_1-j} + \sum_{i=0}^{T_1-1} \frac{1}{s_1-i} \right]. \end{aligned} \tag{61}$$

The first order term in $\frac{k_2}{k_1}$ vanishes and finally

$$\begin{aligned} \bar{\tau}(T_1,T_2) &\approx \sum_{i=0}^{T_1-1} \frac{1}{k_1 m(s_1-1)} \\ &\approx \frac{1}{k_1 m} \log\left(\frac{s_1}{s_1-T_1} \right) \quad \text{when} \quad k_1 \gg k_2, \quad m \gg 1. \end{aligned} \tag{62}$$

This result can be compared to formula (51), obtained for a single threshold and a uniform killing rate. In both cases, we recover the zero order approximation of Eq. (19), which relies asymptotically on MS_1 properties (and not the killing effect or the number of $|MS_2|$ bindings).

V. CONCLUSION AND PERSPECTIVES

We have presented here a general approach to estimate the mean time to form exactly T chemical bonds between substrate and ligand particles. The present computation is based on a Markovian model, which allows us to obtain explicit formula, confirmed by Brownian simulations. Interestingly, the MTT is a nonlinear function T. This result may have other several consequences in cellular biology, including the design of molecular switches [20] where upon the activation of a molecular threshold, the cell fate can be reprogrammed. Another example is the nonlinear relation between the number Calmodulin Kinase II molecules and the induction of long term potentiation [4] in synaptic plasticity. A final example is the spindle checkpoint occurring during the cell mitosis [21]: indeed during metaphase, centrosome nucleated microtubules interact with the chromosome kinetochores to build the mitotic spindle. Only after all chromosomes have become aligned at the metaphase plate, when every kinetochore is properly attached to a bundle of microtubules, does the cell enter anaphase. Our analysis can be used to estimate the probability and the mean time of the number of bindings occurring between kinetochores (the number of which is equal to that of chromosomes ranging from a few to less than 50) and the anaphase activators (our M-molecule). Finally, the present approach disregards the target organization in the cellular domain, which may have a significant effect, especially when the targets can cluster [17–19]

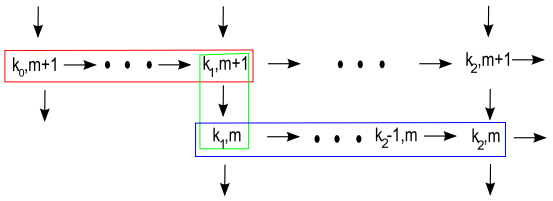


FIG. 5. (Color online) The Markov diagram for a path going from $(k_0,m+1)$ to (k_2,m).

APPENDIX A

We prove relation (34). Indeed, the formula is true for $k=1$ or $m=M-1$ and a direct computation shows it satisfies (31). We obtain the formula, by considering Eq. (31): $q_{k,m}$ can be expressed as a sum of two contributions, one depending on $q_{k-1,m}$ and the other on $q_{k,m+1}$. Thus for $k<k'$ and $m'<m$, we can express $q_{k',m'}$ as a function of $q_{k,m}$, by summing the contribution of $q(k',m')$ in the diagram of Fig. 2 for all "paths" leading from (k,m) to (k',m'). In Fig. 5, we represent a possible path from $(k_0,m+1)$ to (k_2,m), when the killing happens at state $(k_1,m+1)$.

The contribution of $q_{k_1,m}$ to $q_{k_2,m}$ is obtained by using inductively (31) (left box in Fig. 5). Thus

$$\begin{aligned} q_{k_2,m} = &\frac{q_{k_1,m}\lambda(S-k_1)(m-k_1)}{[\lambda(S-k_2)+\mu](m-k_2)} \prod_{i=k_1+1}^{k_2-1} \frac{\lambda(S-i)}{\lambda(S-i)+\mu} \\ &+ (\text{terms not depending on } q_{k_1,m}), \end{aligned} \tag{A1}$$

where the terms that do not depend on $q_{k_1,m}$ will arise from the summation of $k,m+r$ where $r \geq 1$ that will be taken off later on. The contribution of $q_{k_1,m+1}$ to $q_{k_1,m}$ (middle box in Fig. 5) is given by

$$q_{k_1,m} = \frac{\mu}{\lambda(S-k_1)+\mu} q_{k_1,m+1} + \frac{\lambda(S-k_1+1)}{\lambda(S-k_1)+\mu} q_{k_1-1,m}. \tag{A2}$$

Similarly to Eq. (A1), we get the contribution of $q_{k_0,m+1}$ to $q_{k_1,m+1}$ (red box in Fig. 5). Finally, the expression for the

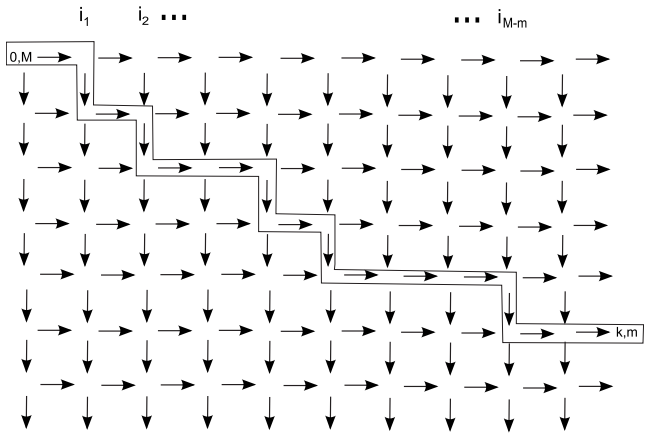


FIG. 6. Markov diagram for a path starting at $(0,M)$ and ending at (k,m). There are $k+M-m$ steps going down $M-m$ times and going forward k times.

contribution of the path represented in Fig. 5 is

$$q_{k_2,m} = \frac{\mu\lambda(S-k_0)(m-k_0)}{[\lambda(S-k_1)+\mu][\lambda(S-k_2)+\mu](m-k_2)} \times \left[\prod_{i=k_0+1}^{k_2-1} \frac{\lambda(S-i)}{\lambda(S-i)+\mu}\right] q_{k_0,m+1} + \text{sum of other contributions}. \quad \text{(A3)}$$

We obtain all the contributions by summing over all possible k_1 in the diagram, i.e., all $k_0 \le k_1 \le k_2$ and we obtain

$$q_{k_2,m} = \left[\sum_{i=k_0}^{k_2} \frac{\mu}{\lambda(S_0-i)+\mu}\right] \frac{\lambda(S-k_0)(m-k_0)}{[\lambda(S-k_2)+\mu](m-k_2)} \times \left[\prod_{i=k_0+1}^{k_2-1} \frac{\lambda(S-i)}{\lambda(S-i)+\mu}\right] q_{k_0,m+1}. \quad \text{(A4)}$$

More generally, by summing over all the contributions, we can obtain an expression for $q_{k,m}$ as a function of $q_{0,M}$: each contribution in the diagram corresponds to a certain path going down $M-m$ times and moving k times forward (see Fig. 6). The path goes down on the diagram at $i_1, .. i_{M-m}$ where $0 \le i_1 \le \ldots \le i_{M-m} \le k$. When summing over all configurations (i_j), we obtain

$$q_{k,m} = \left[\sum_{0\le i_1,\ldots,i_{M_0-m}\le k} \prod_{i_j} \frac{\mu}{\lambda(S-i_j)+\mu}\right] \times \left[\prod_{i=0}^{k-1} \frac{\lambda(S-i)}{\lambda(S-i)+\mu}\right] \frac{M(\lambda S+\mu)}{[\lambda(S-k)+\mu](m-k)} q_{0,M}. \quad \text{(A5)}$$

Using that $q_{0,M}=1/(M(\lambda S+\mu))$ [Eq. (26)], we obtain the formula (34).

APPENDIX B

We prove here relation (42) is equal to Eq. (43): $\forall M \ge T \ge 1$

$$X^T \sum_{k=0}^{M-T} \binom{T-1+k}{k} (1-X)^k = \sum_{k=T}^{M} \binom{M}{k} X^k (1-X)^{M-k}. \quad \text{(A6)}$$

Equivalently, we have to show (replace X by $1-X$, divide by X^T and replace k by $k-T$ on right side)

$$\sum_{k=0}^{M-T} \binom{T-1+k}{k} X^k = \sum_{k=0}^{M-T} \binom{M}{k+T} X^{M-k-T}(1-X)^k. \quad \text{(A7)}$$

We prove (A7) by induction: for $T=1$ (A7) is true for all M and for all M it is true for $T=M-1$.

Suppose for all T that it is true for $M-1 \ge T$, i.e.,

$$\sum_{k=0}^{M-1-T} \binom{T-1+k}{k} X^k = \sum_{k=0}^{M-1-T} \binom{M-1}{k+T} X^{M-1-k-T}(1-X)^k, \quad \text{(A8)}$$

and let us prove (A8) for M: First, we rewrite the left-hand side of Eq. (A7)

$$\sum_{k=0}^{M-T} \binom{T-1+k}{k} X^k = \sum_{k=0}^{M-1-T} \binom{T-1+k}{k} X^k + \binom{M-1}{M-T} X^{M-T}. \quad \text{(A9)}$$

Second, we consider the right-hand side

$$\begin{aligned} &\sum_{k=0}^{M-T} \binom{M}{k+T} X^{M-k-T}(1-X)^k \\ &= \sum_{k=1}^{M-T} \binom{M}{k+T} X^{M-k-T}(1-X)^k + \binom{M}{T} X^{M-T} \\ &= \sum_{k=0}^{M-T-1} \binom{M}{k+T+1} X^{M-k-1-T}(1-X)^{k+1} + \binom{M}{T} X^{M-T}. \end{aligned} \quad \text{(A10)}$$

Using Pascal's rule,

$$= (1-X)^{M-T} + \binom{M}{T} X^{M-T} + (1-X) \times \sum_{k=0}^{M-T-2} \left[\binom{M-1}{k+T} + \binom{M-1}{k+T+1}\right] X^{M-k-1-T}(1-X)^k, \quad \text{(A11)}$$

$$= (1-X)^{M-T} + \binom{M}{T} X^{M-T} + \sum_{k=0}^{M-T-2} \binom{M-1}{k+T} X^{M-k-1-T}(1-X)^k - X \sum_{k=0}^{M-T-2} \binom{M-1}{k+T} X^{M-k-1-T}(1-X)^k + \sum_{k=0}^{M-T-2} \binom{M-1}{k+T+1} X^{M-k-1-T}(1-X)^{k+1}, \quad \text{(A12)}$$

$$= \sum_{k=0}^{M-T-1} \binom{M-1}{k+T} X^{M-k-1-T}(1-X)^k - X \sum_{k=0}^{M-T-2} \binom{M-1}{k+T} X^{M-k-1-T}(1-X)^k + \sum_{k=0}^{M-T-2} \binom{M-1}{k+T+1} X^{M-k-1-T}(1-X)^{k+1} + (1-X)^{M-T} + \binom{M}{T} X^{M-T} - (1-X)^{M-T-1}, \quad \text{(A13)}$$

$$= \sum_{k=0}^{M-T-1} \binom{M-1}{k+T} X^{M-k-1-T}(1-X)^k - X \sum_{k=0}^{M-T-2} \binom{M-1}{k+T} X^{M-k-1-T}(1-X)^k + \sum_{k=0}^{M-T-3} \binom{M-1}{k+T+1} X^{M-k-1-T}(1-X)^{k+1} + X(1-X)^{M-T-1} + (1-X)^{M-T} + \binom{M}{T} X^{M-T} - (1-X)^{M-T-1}, \quad \text{(A14)}$$

$$= \sum_{k=0}^{M-T-1} \binom{M-1}{k+T} X^{M-k-1-T}(1-X)^k - \sum_{k=0}^{M-T-2} \binom{M-1}{k+T} X^{M-k-T}(1-X)^k + \sum_{k=1}^{M-T-2} \binom{M-1}{k+T} X^{M-k-T}(1-X)^k + \binom{M}{T} X^{M-T}, \quad \text{(A15)}$$

$$= \sum_{k=0}^{M-T-1} \binom{M-1}{k+T} X^{M-k-1-T}(1-X)^k + \binom{M}{T} X^{M-T} - \binom{M-1}{T} X^{M-T}, \quad \text{(A16)}$$

$$= \sum_{k=0}^{M-T-1} \binom{M-1}{k+T} X^{M-k-1-T}(1-X)^k + \binom{M-1}{T-1} X^{M-T}. \quad \text{(A17)}$$

By induction, and with Eq. (A9) it follows

$$\sum_{k=0}^{M-T} \binom{T-1+k}{k} X^k = \sum_{k=0}^{M-T} \binom{M}{k+T} X^{M-k-T}(1-X)^k. \quad \text{(A18)}$$

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