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Distributions of lifetime and maximum size of abortive clathrin-coated pits

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Abstract

Clathrin-mediated endocytosis is a complex process through which eukaryotic cells internalize nutrients, antigens, growth factors, pathogens etc. The process occurs via the formation of invaginations on the cell membrane, called clathrin-coated pits (CCPs). Over the years much has been learned about the mechanism of CCP assembly, but a complete understanding of the assembly process still remains elusive. In recent years, using fluorescence microscopy, studies have been done to determine the statistical properties of CCP formation. In this paper, using a recently proposed coarse-grained, stochastic model of CCP assembly (Biophys. J. **102**, 2725 (2012)), we suggest new ways of analyzing such experimental data. To be more specific, we derive analytical expressions for the distribution of maximum size of abortive CCPs, and the probability density of their lifetimes. Our results show how these functions depend on the kinetic and energetic parameters characterizing the assembly process, and therefore could be useful in extracting information about the mechanism of CCP assembly from experimental data. We find excellent agreement between our analytical results and those obtained from kinetic Monte Carlo simulations of the assembly process.

I. INTRODUCTION

Clathrin-mediated endocytosis (CME) is a process of vesicle formation at the cell plasma membrane (PM).^{1–3} It has many functions, including bringing nutrients into the cell, regulating the number of receptors on the cell surface, and recycling the contents of synaptic vesicles at the nerve terminals. CME starts with the self-assembly of specialized domains on the cytoplasmic side of PM, called clathrin-coated pits (CCPs). The CCPs grow in size, bind cargo (molecules to be internalized), and finally pinch off from the PM to form transport vesicles called clathrin-coated vesicles (CCVs). The CCVs move inside the cell and fuse with early endosomes where cargo is sorted to various intracellular compartments.

The formation of a CCP is a highly complex, dynamic process which involves the recruitment of a large number of proteins onto the membrane.^{2–4} These proteins interact with each other to form a highly structured protein coat. Typically, the most abundant proteins in the coat are clathrin – a triskelial shaped molecular complex – and its partner adaptor protein AP-2. The AP-2 molecules aid the assembly of clathrin triskelions into a

lattice^{5,6}, which participates in membrane bending⁷. The coat also includes other membrane bending proteins which, in cooperation with the clathrin lattice, cause the membrane to invaginate.^{8–11}

CCP self-assembly has been a subject of interest for many years. Imaging techniques like electron microscopy, X-ray crystallography and cryo-microscopy provide snapshots of this highly dynamic process, but cannot shed light on the kinetics of vesicle formation. Recently, evanescent-wave microscopy coupled with sophisticated automated image analysis has appeared as a new method that makes it possible to study the dynamics of CCP formation.^{12–14} In these experiments, live cells expressing fluorescent clathrin are observed under the microscope, and the CCPs appear as fluorescent spots. By measuring the variation of fluorescence intensity of individual CCPs as a function of time, researchers can collect statistical information on various aspects of CCP dynamics, including their lifetime, size, and mobility.^{12–14}

The dynamics of CCP assembly, observed through fluorescence microscopy, shows remarkable variability. A fraction of CCPs, called “productive”, grow in size to form CCVs. The size of CCVs typically vary between 20–60 nm in radius.¹⁵ Depending on its size, a CCV contains between 60–150 clathrin triskelion¹⁵, and the entire process, starting from the initiation of a CCP to the formation of a CCV, takes somewhere between 30 seconds to greater than 120 seconds.¹³ In contrast, a large fraction of CCPs, called “abortive”, grow only up to intermediate sizes and eventually disassemble. Such CCPs are short lived and their mean lifetime is less than 20 seconds.¹³ Experiments show that the fate of a CCP is determined by cargo binding. The CCPs which bind sufficient amount of cargo are productive, and CCPs which fail to do so are abortive.^{12,16}

As mentioned earlier, using fluorescence microscopy, researchers can collect statistical information on various aspects of CCP assembly.^{12–14} This statistical approach has opened a new avenue towards studying the dynamics of CCP formation, but the complexity of the phenomena makes it difficult to extract information about the assembly mechanism from such experimental data. In such a situation, simple models that capture the global behavior of the complex phenomena are very useful. To this end, in a recent paper¹⁷, we developed a coarse-grained model of CCP formation to address questions related to the temporal heterogeneity observed in CCP assembly. A key step of our analysis was the mapping of CCP assembly dynamics onto a one-dimensional random walk. Using kinetic Monte Carlo simulations based on the model, we were able to explain the lifetime distribution of abortive pits, the relation between cargo binding and CCP fate, and the wide distribution in lifetimes of productive CCPs.

Our model of CCP assembly can be further used to examine properties of the assembly process, which can be studied experimentally. Focus of the present paper is on analytical studies of the statistical properties of the lifetimes and sizes of the abortive CCPs. The paper is organized as follows. In section II we briefly describe our model of CCP assembly. In sections III and IV we derive analytical solutions for the distribution of maximum size of abortive CCPs and the probability density of their lifetimes, respectively. We discuss the probability densities of lifetimes of abortive pits of different maximum size in section V. In

each section we compare our analytical results with those obtained from kinetic Monte Carlo simulations. Random walk models similar to ours have been used to describe other biological phenomena as well. Examples include the translocation of small solutes through membrane channels^{18,19} and movement of molecular motors on microtubules.²⁰ Thus our results might be useful in studies of other cell physiological processes as well.

II. MODEL

As mentioned earlier, a CCP is a multicomponent system that includes lipids and a large number of proteins (see Fig. 1). Modeling the self-assembly of such a complex entity at molecular level is an impossible task. At best, in such a case one can study a reduced system or take a coarse grained approach. Recently, we proposed a simple coarse-grained model of CCP assembly that captures the main features of the process.¹⁷ We postulated that the protein coat is made up of basic structural units which we refer to as “monomers” (see Fig. 1). The number of monomers is taken to be equal to the number of clathrin molecules, and it is assumed that the properties of the coat formed by the assembly of monomers mimics the properties of the real protein coat. Given these assumptions, it is possible to reduce the complex problem of CCP assembly to a tractable problem of assembly of monomers.

Figure 2 shows the kinetic scheme of the assembly of our model-CCP, which, from now on, we refer to as a “pit”. The number n represents the number of monomers in a pit, and N is the number of monomers in a vesicle. As mentioned earlier the number of clathrin molecules in a CCV is typically between 60 and 140.¹⁵ With this in mind we keep the vesicle size fixed at $N = 100$. Assembly of a new pit starts from site $n = 1$, which represents a single monomer bound to the plasma membrane. After initiation, a pit grows by reversible binding of free monomers (see Figs. 1 and 2). The assembly process eventually ends at site 0 or $N + 1$, where the site $N + 1$ corresponds to a free vesicle with N monomers (not a pit with $N + 1$ monomers). The pits that end up at site 0 correspond to abortive CCPs and the ones that end up at site $N + 1$ correspond to productive CCPs. Thus the model described above maps the pit growth process onto a one-dimensional nearest-neighbor continuous-time random walk between absorbing left and right boundaries (sites 0 and $N + 1$, respectively), with site-dependent transition rates.

The rate constant α_n and β_n describe the growth and decay rates of a pit of size n . We choose the forward rate constants to be of the form $\alpha_n = \gamma f(n)$, $n = 1, 2, \dots, N - 1$, where γ is a constant and function $f(n)$ gives the number of available binding sites on the edge of a pit of size n . By assuming that the pit grows with a constant curvature, and that its shape is always a spherical cap (part of a sphere), the function $f(n)$ can be written in the form

$$f(n) = d \sqrt{n(N - n)}, \quad (1)$$

where the dimensionless parameter $d = 0.19$ is related to the average span of a monomer on the edge of a pit. By fitting the data on lifetimes of CCPs¹³ we were able to determine $\gamma = 0.18 \text{ sec}^{-1}$. The rate constant α_N describes the rate of scission of a vesicle from the membrane. For simplicity, we choose $\alpha_N = \infty$.

We assume that the forward and backward rate constants are related to each other through detailed balance

$$\alpha_{n-1} \exp[-\tilde{F}(n-1)] = \beta_n \exp[-\tilde{F}(n)], \quad n=2, 3, \dots, N, \quad (2)$$

where $\tilde{F}(n) = F(n)/k_B T$, and $F(n)$ is the free energy of the pit formation, which is the change in system free energy due to the disassembly of a pit of size n , measured in units of $k_B T$, where k_B is the Boltzmann constant and T is the absolute temperature. The rate constant β_1 , which describes the rate at which a single monomer dissociates from the cell membrane, is not determined by detailed balance. We choose $\beta_1 = 0.35 \text{ sec}^{-1}$.

The free energy of CCP formation, $F(n)$, includes: (a) energy cost of bending the cell membrane, (b) energy cost of bending the protein coat, (c) stabilizing energy of bonds formed between various proteins and proteins and lipids, and (d) entropic cost of immobilizing the proteins. The free energy can be written in the form (see Ref. 17 for details)

$$F(n) = \Omega n + \Gamma \sqrt{n(N-n)} \quad (3)$$

The first term in Eq. (3), which is proportional to n , includes contributions from (a), (b), (c) and (d). The use of a linear approximation for the binding energy results in the overestimation of its magnitude, so the second term has to be added as a correction. This term is proportional to the length of the edge of the pit, and thus is similar to a line tension energy.²¹ The parameters Ω and Γ have units of energy and their values depend on several system parameters describing the cell membrane and the protein coat.¹⁷ By fitting the data on lifetimes of abortive CCPs we were able to determine $\Omega = 0.22 kT$ and $\Gamma = 0.2 kT$. Figure 3 shows the plot of the free energy for CCPs without cargo. As mentioned earlier, such CCPs are mainly abortive. The free energy increases with pit size and reaches a maximum at $N = n^*$. This free energy barrier, which a growing pit (without cargo) must overcome to form a vesicle is very high. As a consequence, the fraction of cargoless pits that evolve into productive pits is negligibly small.

In Ref. 17 we used the parameter values mentioned in this section (i.e., $N = 100$, $\gamma = 0.18 \text{ sec}^{-1}$, $d = 0.19$, $\beta_1 = 0.35 \text{ sec}^{-1}$, $\alpha_N = 1$, $\Omega = 0.22 k_B T$ and $\Gamma = 0.2 k_B T$). We here use the same values for our kinetic Monte Carlo simulation of the assembly process. The simulation algorithm is discussed in the Appendix.

III. DISTRIBUTION OF MAXIMUM SIZE OF ABORTIVE PITS

Having described how we mapped the dynamics of CCPs onto a one-dimensional random walk, we now calculate the distribution of maximum size of abortive pits. As mentioned earlier, according to our model the abortive pits correspond to the random walkers that are eventually absorbed at site $n = 0$.

Consider an ensemble of random walkers (RWs) that start from an intermediate site n , where $0 < n < N + 1$. After following different individual paths, the RWs eventually reach site $N +$

1 or 0, where they are absorbed. Let $W_{N+1}(n)$ and $W_0(n)$ denote the fractions of RWs that are absorbed at these sites, respectively. These quantities, which in the random walk literature are referred to as the splitting probabilities^{22,23}, satisfy $W_{N+1}(n) + W_0(n) = 1$. The formulas for the splitting probabilities are well known.²³ For $n = 1$ they are

$$W_{N+1}(1) = \frac{\Psi_1}{\Psi_1 + \sum_{m=1}^N \Phi_m}, \quad W_0(1) = 1 - W_{N+1}(1) = \frac{\sum_{m=1}^N \Phi_m}{\Psi_1 + \sum_{m=1}^N \Phi_m}, \quad (4)$$

where $\Psi_n = \exp[\tilde{F}(n)]/\beta_n$ and $\Phi_n = \exp[\tilde{F}(n)]/\alpha_n$.

Now let us consider the RWs that are absorbed at site $n = 0$. Let $M \leq N$ denote the site farthest from site 1 that a RW reaches before being trapped at site 0. M is a random variable. Its distribution is identical to that of the maximum size of abortive pits. Substituting M and $M + 1$ for $N + 1$ in Eq. 4, we see that $W_M(1)$ and $W_{M+1}(1)$ are the fractions of RWs that are absorbed at sites M and $M + 1$, respectively. Thus $W_M(1) - W_{M+1}(1)$ is precisely the fraction of RWs that reach site M and then return back to site 0, without ever reaching site $M + 1$. Consequently, $P(M)$, defined as the probability distribution of the maximum size M , can be written as

$$P(M) = \frac{W_M(1) - W_{M+1}(1)}{W_0(1)} = \frac{1}{W_0(1)} \frac{\Psi_1 \Phi_M}{\left[\Psi_1 + \sum_{m=1}^{M-1} \Phi_m \right] \left[\Psi_1 + \sum_{m=1}^M \Phi_m \right]}. \quad (5)$$

Using the relation $\sum_{M=1}^N [W_M(1) - W_{M+1}(1)] = W_0(1)$, one observes that $P(M)$ is normalized to unity, $\sum_{M=1}^N P(M) = 1$.

The above result has a simple interpretation. The fraction of RWs that have maximum size M is equal to the product of $W_M(1)$, the fraction of RWs that reach site M starting from site 1, and $W_0(M)$, the fraction of RWs that reach site 0 starting from site M , on condition that site $M + 1$ is absorbing. Using Eq. (4) these fractions can be written as

$$W_M(1) = \frac{\Psi_1}{\Psi_1 + \sum_{m=1}^{M-1} \Phi_m}, \quad W_0(M) = \frac{\Phi_M}{\Psi_1 + \sum_{m=1}^M \Phi_m}. \quad (6)$$

The second formula in Eq. (6) is obtained using a symmetry argument. Then, using correct normalization, the distribution of maximum size can be written as $P(M) = W_M(1)W_0(M)/W_0(1)$, which gives the same result as in Eq. (5).

Figure 4 shows the distribution of maximum size of abortive pits, M , calculated from Eq.(5), compared with the results of kinetic Monte Carlo simulations. One can see very good agreement between the analytical and numerical results. The function $P(M)$ decreases

rapidly with increasing values of M , which means that most of the abortive pits grow to very small sizes (much smaller than the critical size n^*). Sizes of abortive CCPs seen during experiments show qualitatively similar behavior.¹³

IV. PROBABILITY DENSITY OF LIFETIME OF ABORTIVE PITS

The probability density of the lifetime of abortive pits is identical to that of the RWs that are absorbed at $n = 0$. To find the latter we first calculate, $\tau(M)$, the mean lifetime of RWs which reach a maximum size M . This time can be written as

$$\tau(M) = \tau(1, M|0) + \tau(M, 0|M+1), \quad (7)$$

where $\tau(1, M|0)$ corresponds to the conditional mean first passage time from site 1 to site M , without ever reaching site $n = 0$. Similarly, $\tau(M, 0|M+1)$ corresponds to the conditional mean first passage time from site M to site 0, without ever reaching site $M+1$. The formulas for the conditional mean first passage times are well known²³ and given by

$$\tau(1, M|0) = \frac{\sum_{m=1}^{M-1} \exp(-F_m) \sum_{l=1}^m \Psi_l \sum_{l=m}^{M-1} \Phi_l}{\Psi_1 + \sum_{m=1}^{M-1} \Phi_m}, \quad (8)$$

$$\tau(M, 0|M+1) = \frac{\sum_{m=1}^M \exp(-F_m) \sum_{l=1}^m \Psi_l \sum_{l=m}^M \Phi_l}{\Psi_1 + \sum_{m=1}^M \Phi_m}. \quad (9)$$

Figure 5 shows a comparison between the values of $\tau(M)$ calculated from Eq.(7) and from simulations. The analytical results agree well with the simulation results.

Next, we proceed to the probability density of lifetime of abortive pits, $\phi(t)$. Let $\phi(t|M)$ be the probability density of the lifetime of abortive pits, on condition that the maximum size of the pits is M . Then $\phi(t)$ can be written as

$$\phi(t) = \sum_{M=1}^N \phi(t|M) P(M). \quad (10)$$

A general solution for $\phi(t|M)$ is not known. In view of this circumstance we use the mean field approximation,

$$\phi(t|M) = \delta(t - \tau(M)), \quad (11)$$

which leads to

$$\phi(t) = \sum_{M=1}^N \delta(t - \tau(M)) P(M). \quad (12)$$

Notice that $\phi(t)$ satisfies the normalization condition $\int_0^\infty \phi(t) dt = 1$.

Figure 6 shows the plot of the lifetime distribution of abortive pits calculated using Eq. (12), compared with the results of Monte Carlo simulation. When plotting the analytical result we approximate the delta functions in Eq. (12) by rectangular functions normalized to unity

$$\delta(t - \tau(M)) = \frac{2}{\tau(M+1) - \tau(M-1)} \Pi \left[\frac{\tau(M-1) + \tau(M)}{2}, \frac{\tau(M) + \tau(M+1)}{2} \right], \quad M > 1 \quad (13)$$

$$\delta(t - \tau(1)) = \frac{2}{\tau(2) + \tau(1)} \Pi \left[0, \frac{\tau(2) + \tau(1)}{2} \right], \quad (14)$$

where $\Pi(x, y)$ is the rectangular function which takes the value 1 between x and y ($x < y$), and zero otherwise. As shown in the next section, for small values of M the probability density $\phi(t|M)$ is localized. Thus an approximation with rectangular functions is reasonable, resulting in good agreement between theory and simulations at short times (where the main contribution to $\phi(t)$ comes from small M). As M increases, the probability density $\phi(t|M)$ becomes increasingly diffusive. As a consequence, the mean-field approximation becomes less accurate. This is, presumably, the reason for the difference between theory and simulations at large times, where the main contribution to $\phi(t)$ comes from large M . In the following section we present a method to calculate the Laplace transform of $\phi(t|M)$.

V. CONDITIONAL LIFETIME PROBABILITY DENSITY $\phi(t|M)$

Since the function $P(M)$ decreases sharply with increasing value of M (see Fig. 4), the main contribution to $\phi(t)$ comes from the first few terms on the right-hand side of Eq. (10). As mentioned earlier, a general solution for the conditional lifetime probability density, $\phi(t|M)$, is not known; however, its analytical form for the first few values of M can be calculated. In this section we derive $\phi(t|M)$ for $M = 1$ and its Laplace transform $\hat{\phi}(s|M)$, for $M = 2$ and 3. The approach we use is easily generalizable to larger values of M .

Consider an ensemble of RWs that start at site 1 at time $t = 0$. At each instant of time, some RWs jump to site 0, and some to site 2. Let $S(t|1)$ be the probability that a RW does not escape from site 1 up to time t . This survival probability satisfies

$$\dot{S}(t|1) = -k_1 S(t|1), \quad k_1 = \alpha_1 + \beta_1. \quad (15)$$

Solving the above equation with the initial condition $S(0|1) = 1$, we find

$$S(t|1) = \exp(-k_1 t). \quad (16)$$

Let $f_{1 \rightarrow 0}(t)$ be the probability flux formed by the RW trajectories that start from site 1 at $t = 0$ and enter site 0 at time t , without ever having jumped to site 2. The flux is given by

$$f_{1 \rightarrow 0}(t) = \beta_1 S(t|1) = \beta_1 \exp(-k_1 t). \quad (17)$$

The time integral of the flux from zero to infinity is the fraction of trajectories that start from site 1 and end up at site 0 without visiting other sites,

$$\int_0^\infty f_{1 \rightarrow 0}(t) dt = \beta_1 / k_1. \quad (18)$$

The conditional probability density that this passage occurs at time t , i.e., $\phi(t|1)$, is by definition the ratio of the flux $f_{1 \rightarrow 0}(t)$, Eq. (17), to the integral of flux, Eq. (18),

$$\phi(t|1) = \frac{f_{1 \rightarrow 0}(t)}{\int_0^\infty f_{1 \rightarrow 0}(t) dt} = k_1 \exp(-k_1 t). \quad (19)$$

Next, to find the conditional probability density $\phi(t|2)$, we consider those realizations of RW trajectories which start from site 1 at $t = 0$ and enter site 0 at time t , on condition that they visited site 2 at least once and never visited site 3. The lifetime of a RW that follows such a trajectory can be divided into two parts: time t' taken to make the first transition from site 1 to site 2, $t' < t$, and time $t - t'$ taken to make the transition from site 2 to site 0. Then we can write $\phi(t|2)$ as a convolution

$$\phi(t|2) = \int_0^t \phi_{1 \rightarrow 2}(t') \phi_{2 \rightarrow 0}(t - t') dt', \quad (20)$$

where $\phi_{1 \rightarrow 2}(t)$ and $\phi_{2 \rightarrow 0}(t)$ are the conditional probability densities of the first passage times from site 1 to 2 and from site 2 to 0, respectively. Following the same reasoning used to derive $\phi(t|1)$ in Eq. (19), we find

$$\phi_{1 \rightarrow 2}(t) = \phi(t|1) = k_1 \exp(-k_1 t). \quad (21)$$

To derive $\phi_{2 \rightarrow 0}(t)$, consider those realizations of RW trajectories which start from site 2 at time $t = 0$ and enter site 0 at time t , on condition that they never jumped to site 3. The probability flux formed by such trajectories, $f_{2 \rightarrow 0}(t)$, is given by

$$f_{2 \rightarrow 0}(t) = \beta_1 G_{12}^{(2)}(t), \quad (22)$$

where $G_{ij}^{(2)}(t)$, $i, j = 1, 2$, is the propagator (Green's function), which is the probability of finding the RW at site i at time t , on condition that it starts from site j at time $t = 0$. This propagator describes the dynamics of the two state system (as indicated by the superscript symbol (2)) and satisfies

$$\dot{G}_{1j}^{(2)}(t) = -k_1 G_{1j}^{(2)}(t) + \beta_2 G_{2j}^{(2)}(t), \quad (23)$$

$$\dot{G}_{2j}^{(2)}(t) = \alpha_1 G_{1j}^{(2)}(t) - k_2 G_{2j}^{(2)}(t), \quad (24)$$

where $k_2 = \alpha_2 + \beta_2$, with the initial condition $G_{ij}^{(2)}(0) = \delta_{ij}$. Solving these equations in Laplace space, we find that the Laplace transform of the flux $f_{2 \rightarrow 0}(t)$ is given by

$$\hat{f}_{2 \rightarrow 0}(s) = \frac{\beta_1 \beta_2}{(s+k_1)(s+k_2) - \alpha_1 \beta_2}, \quad (25)$$

where s is the Laplace parameter. (The Laplace transform $\hat{F}(s)$ of a function $F(t)$ is defined as $\hat{F}(s) = \int_0^\infty \exp(-st) F(t) dt$.) Again, by definition the conditional probability density $\phi_{2 \rightarrow 0}(t)$ and its Laplace transform $\hat{\phi}_{2 \rightarrow 0}(s)$ can be written as

$$\phi_{2 \rightarrow 0}(t) = \frac{f_{2 \rightarrow 0}(t)}{\int_0^\infty f_{2 \rightarrow 0}(t) dt}, \quad (26)$$

$$\hat{\phi}_{2 \rightarrow 0}(s) = \frac{\hat{f}_{2 \rightarrow 0}(s)}{\int_0^\infty f_{2 \rightarrow 0}(t) dt} = \frac{\hat{f}_{2 \rightarrow 0}(s)}{\hat{f}_{2 \rightarrow 0}(s=0)}. \quad (27)$$

Substituting the result of Eq. (25) in the above equation, we get

$$\hat{\phi}_{2 \rightarrow 0}(s) = \frac{k_1 k_2 - \alpha_1 \beta_2}{(s+k_1)(s+k_2) - \alpha_1 \beta_2}. \quad (28)$$

Using the above equation and the Laplace transform of $\phi_{1 \rightarrow 2}(t)$, Eq. (21), we eventually find the Laplace transform of $\phi(t|2)$, Eq. (20),

$$\hat{\phi}(s|2) = \hat{\phi}_{1 \rightarrow 2}(s) \hat{\phi}_{2 \rightarrow 0}(s) = \frac{k_1 (k_1 k_2 - \alpha_1 \beta_2)}{(s+k_1) [(s+k_1)(s+k_2) - \alpha_1 \beta_2]}. \quad (29)$$

Next we use the same approach to find $\phi(t|3)$. Now we consider those realizations of RW trajectories which start from site 1 at $t = 0$ and enter site 0 at time t , on condition that they visit site 3 at least once and never visit site 4. The lifetime of such trajectories can again be divided into two parts: time t' taken to make the first transition from site 1 to site 3, $t' < t$, and time $t - t'$ taken to make the transition from site 3 to site 0. Then the conditional probability density $\phi(t|3)$ and its Laplace transform $\hat{\phi}(s|3)$ can be written as

$$\phi(t|3) = \int_0^t \phi_{1 \rightarrow 3}(t') \phi_{3 \rightarrow 0}(t - t') dt', \quad (30)$$

$$\hat{\phi}(s|3) = \hat{\phi}_{1 \rightarrow 3}(s) \hat{\phi}_{3 \rightarrow 0}(s). \quad (31)$$

where $\varphi_{1 \rightarrow 3}(t)$ and $\varphi_{3 \rightarrow 0}(t)$ are the conditional probability densities of the first passage times from site 1 to 3 and from site 3 to 0, respectively.

By definition, $\varphi_{1 \rightarrow 3}(t)$ and its Laplace transform $\hat{\phi}_{1 \rightarrow 3}(s)$ are given by

$$\phi_{1 \rightarrow 3}(t) = \frac{f_{1 \rightarrow 3}(t)}{\int_0^\infty f_{1 \rightarrow 3}(t) dt}, \quad (32)$$

$$\hat{\phi}_{1 \rightarrow 3}(s) = \frac{\hat{f}_{1 \rightarrow 3}(s)}{\hat{f}_{1 \rightarrow 3}(s=0)}, \quad (33)$$

where $f_{1 \rightarrow 3}(t)$ is the probability flux formed by those RW trajectories that start from site 1 at $t = 0$ and enter site 3 for the first time at time t . The flux is given by

$$f_{1 \rightarrow 3}(t) = \alpha_2 G_{21}^{(2)}(t), \quad (34)$$

where $G_{21}^{(2)}(t)$ is the propagator satisfying Eq. (24) with initial conditions $G_{11}^{(2)}(0)=1$, $G_{21}^{(2)}(0)=0$. Solving the set of equations in Eq. (24), one can find that the Laplace transform of $\varphi_{1 \rightarrow 3}(t)$ is given by

$$\hat{\phi}_{1 \rightarrow 3}(s) = \frac{k_1 k_2 - \alpha_1 \beta_2}{(s+k_1)(s+k_2) - \alpha_1 \alpha_2}. \quad (35)$$

Continuing, the probability density $\varphi_{3 \rightarrow 0}(t)$ and its Laplace transform are defined as

$$\phi_{3 \rightarrow 0}(t) = \frac{f_{3 \rightarrow 0}(t)}{\int_0^\infty f_{3 \rightarrow 0}(t) dt}, \quad (36)$$

$$\hat{\phi}_{3 \rightarrow 0}(s) = \frac{\hat{f}_{3 \rightarrow 0}(s)}{\hat{f}_{3 \rightarrow 0}(s=0)}, \quad (37)$$

where $f_{3 \rightarrow 0}(t)$ is the probability flux formed by those RW trajectories that start at site 3 at $t = 0$ and enter site 0 at time t without entering site 4 during this time. The flux can be written as

$$f_{3 \rightarrow 0}(t) = \beta_1 G_{13}^{(3)}(t), \quad (38)$$

where $G_{ij}^{(3)}(t)$, $i, j = 1, 2, 3$, is the three-state propagator satisfying

$$\dot{G}_{1j}^{(3)}(t) = -k_1 G_{1j}^{(3)}(t) + \beta_2 G_{2j}^{(3)}(t), \quad (39)$$

$$\dot{G}_{2j}^{(3)}(t) = \alpha_1 G_{1j}^{(3)}(t) - k_2 G_{2j}^{(3)}(t) + \beta_3 G_{3j}^{(3)}(t), \quad (40)$$

$$\dot{G}_{3j}^{(3)}(t) = \alpha_2 G_{2j}^{(3)}(t) - k_3 G_{3j}^{(3)}(t), \quad (41)$$

where $k_3 = \alpha_3 + \beta_3$, with the initial condition $G_{ij}^{(3)}(0) = \delta_{ij}$. Solving the set of equations in Eq. (41) in Laplace space, we obtain $\hat{G}_{13}^{(3)}(s)$. Substituting that result into Eq. (37) we get

$$\hat{\phi}_{3 \rightarrow 0}(s) = \frac{k_1 k_2 k_3 - \alpha_2 \beta_3 k_1 - \alpha_1 \beta_2 k_3}{(s+k_1)(s+k_2)(s+k_3) - \alpha_2 \beta_3 (s+k_1) - \alpha_1 \beta_2 (s+k_3)}. \quad (42)$$

Using Eqs. (35) and (42) we can eventually find the Laplace transform of the probability density $\phi(t|3)$, Eq. (31).

Figure 7 shows plots of $\phi(t|M)$ for $M = 1, 2$ and 3 . The solid curves were drawn using Eq. (19) and the inverse Laplace transform of $\hat{\phi}(s|2)$ and $\hat{\phi}(s|3)$ in Eqs. (29) and (31), respectively. Circles show the values of $\phi(t|M)$ obtained from our kinetic Monte Carlo simulations. One can see excellent agreement between our analytical and numerical results.

The method described above can be used to calculate $\hat{\phi}(s|M)$ for higher values of M as well. The general formula for $\hat{\phi}(s|M)$ has the same structure as those in Eqs. (29) and (31)

$$\hat{\phi}(s|M) = \hat{\phi}_{1 \rightarrow M}(s) \hat{\phi}_{M \rightarrow 0}(s), \quad (43)$$

where $\hat{\phi}_{1 \rightarrow M}(s)$ and $\hat{\phi}_{M \rightarrow 0}(s)$ are the Laplace transforms of the conditional probability densities of the first passage times from site 1 to M and from site M to 0, respectively. They are given by

$$\hat{\phi}_{1 \rightarrow M}(s) = \frac{\hat{G}_{M-1 \ 1}^{(M-1)}(s)}{\hat{G}_{M-1 \ 1}^{(M-1)}(s=0)}, \quad \hat{\phi}_{M \rightarrow 0}(s) = \frac{\hat{G}_{1 \ M}^{(M)}(s)}{\hat{G}_{1 \ M}^{(M)}(s=0)}, \quad (44)$$

where $\hat{G}_{ij}^{(M)}(s)$ is the Laplace transform of the propagator $G_{ij}^{(M)}(t)$ of the corresponding M -state system. As M increases, the expressions for the propagators and the formula for $\hat{\phi}(s|M)$ become increasingly complicated.

VI. CONCLUDING REMARKS

Formation of a clathrin-coated vesicle is a highly dynamic process. Researchers are continuously developing new experimental methods to gain a better understanding of this and related phenomena. However, as new methods and more data become available, new ways of extracting information from the data should be developed. The complexity of the process makes this a very challenging task.

In a previous paper¹⁷ we proposed a stochastic model of CCP assembly which we used to address some questions related to inhomogeneity of lifetimes of abortive and productive CCPs. In this paper we extended that analysis and derived analytical solutions for the probability distribution of the maximum size of abortive pits and the probability density of their lifetimes. These results depend on the parameters describing the structural properties of the CCP components, like the bending rigidity and the spontaneous curvature of the cell membrane and the protein coat, and the rate constants which, apart from other quantities, depend upon the concentration of free monomers.

There are several ways in which these results can be used to gain insights into the complex mechanism of CCP assembly. For example, during the assembly process the coat proteins cooperate with each other to bend the cell membrane (an energetically costly process), the details of which are poorly understood. By fitting the experimental data with our results the values of the coat parameters can be determined. Similarly the formulas for $\varphi(t|M)$ can be used to gain information about the kinetics of early stages of CCP formation.

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Appendix A: Numerical analysis

In our kinetic Monte Carlo simulations we use the kinetic scheme shown in Fig. 2. The forward and backward rate constants were determined using the parameter values and the functions $F(n)$ and $f(n)$ mentioned in section II. We started an ensemble of $6 * 10^6$ pits at $n = 1$ and calculated the time evolution of their size using kinetic Monte Carlo simulation.²⁴ At the end of the simulation we collected all the pits with abortive fate, i.e, the pits that eventually end up at $n = 0$, and used information about their lifetimes and maximum sizes to calculate the quantities of interest.

The presentations of the model and simulation algorithm, in this paper, differ slightly from those appearing in Ref. 17. To make comparison with Ref. 17 easier, we point out the following differences:

- In Ref. 17, for calculation purposes we used the energy function $E(n)$, see Eq. 6 and Fig. 1 in Ref. 17. In this paper we use the free energy $F(n)$. The two are related through $F(n) = E(n) + nkT \ln(cv)$, where c is the concentration free monomers and v is a characteristic volume. The second term corresponds to the entropic cost of immobilizing n monomers. It is obtained by treating the monomers in solution like an ideal gas.
- In Ref. 17, to make our simulations commensurate with the experiments, we started the pits at $n = 5$ and considered a pit abortive if its size reached $n = 4$. Also, we did not include pits with lifetimes less than 2 seconds in our calculations. Since the main emphasis of this paper is to present the analytical formulas, we relax these constraints and use the method described above.

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FIG. 1.

A real CCP is a multicomponent system, made of membrane lipids and a large number of various proteins. Our model coat is described in terms of identical monomeric units.

FIG. 2.
Kinetic scheme of CCP assembly.

FIG. 3.
Free energy of CCP formation for the case of abortive CCPs.

FIG. 4. Probability distribution of the maximum size of abortive pits calculated from Eq.(5), and from simulations.

FIG. 5.

The mean lifetime of abortive pits with fixed maximum size, calculated from Eq.(7) and from simulations.

FIG. 6. Probability density of lifetimes of abortive pits, using Eq. 12 and Monte Carlo simulations.

FIG. 7.
Lifetime distribution of abortive pits on condition that their maximum size is M .