How Does a Virus Bud?

Daniel M. Lerner,* Joshua M. Deutsch,‡ and George F. Oster*
*Graduate Group in Biophysics and Departments of Molecular and Cell Biology and Entomology, University of California, Berkeley, California 94720; ‡Department of Physics, University of California, Santa Cruz, California 95064 USA

ABSTRACT How does a virus bud from the plasma membrane of its host? Here we investigate several possible rate-limiting processes, including thermal fluctuations of the plasma membrane, hydrodynamic interactions, and diffusion of the glycoprotein spikes. We find that for bending moduli greater than 3×10^{-13} ergs, membrane thermal fluctuations are insufficient to wrap the viral capsid, and the mechanical force driving the budding process must arise from some other process. If budding is limited by the rate at which glycoprotein spikes can diffuse to the budding site, we compute that the budding time is 10–20 min, in accord with the experimentally determined upper limit of 20 min. In light of this, we suggest some alternative mechanisms for budding and provide a rationale for the observation that budding frequently occurs in regions of high membrane curvature.

INTRODUCTION

Some animal viruses mature by a process known as budding, in which the nucleocapsid wraps itself in the host cell's plasma membrane and then exits the cell by pinching off from the cell surface. The best understood such virus is the Semliki Forest virus, an alphavirus. Electron micrograph studies have shown that the completed virion has 240 copies of the E1-E2-E3 spike glycoprotein at 80 sites. There are four sites on each triangular face of the icosahedral capsid and a triplet of the E1-E2-E3 trimer is located at each site (1–3). Fig. 1 shows an approximate scale drawing of the viral capsid as it docks with its spike proteins on the plasma membrane. The Sindbis virion is similar except for the absence of the E3 protein.

Simons and Garoff proposed the following model of budding (4). The p62-E1 complex moves to the cell surface from the Golgi where the p62 precursor is cleaved to E2 and E3 resulting in the final E1-E2-E3 glycoprotein. The nucleocapsid is transported to the cell surface and binds to a cluster of spike proteins. The virus capsid wraps itself in the host's membrane by trapping thermal fluctuations: the membrane advances to the next binding site on the capsid when a membrane fluctuation occurs which is large enough to bring a diffusing spike protein to this site, where it binds to a capsid protein and anchors the membrane to the capsid. Vaux et al. (5) have shown that there is a specific binding interaction between the capsid protein and the cytoplasmic tail of the E2 spike protein.

In this paper, we have attempted to determine whether thermal fluctuations are sufficient to drive the budding process by calculating the budding time predicted by the model of Simons et al. Three conditions must be satisfied for the membrane to advance to the next binding site on the capsid: (i) the membrane must experience a large enough fluctuation to bring a capsid binding site in apposition to a spike protein, (ii) water must be squeezed out from between the membrane and the capsid, and (iii) there must be a spike protein available to bind to the capsid protein. We find that membrane fluctuations are frequent enough to account for the observed budding rates only if the bending modulus is less than 3×10^{-13} dyne-cm, which would exclude most experimental measurements on bilayers. In this case, when a spike protein diffuses along the membrane to the capsid, and assuming it then binds almost instantly, the time required for budding is solely dependent on the availability of spike proteins. In this diffusion limited case, we find a budding time of 10–20 min, which is within experimentally determined budding times. However, if the bending modulus is larger than 10^{-13} dyne-cm, thermal fluctuations are insufficient to account for the observed budding times.

CAN THERMAL FLUCTUATIONS WRAP THE VIRUS CAPSID?

In order to compute the time to wrap the viral capsid in the plasma membrane, we first calculate the bending energy required to deform the membrane from one glycoprotein spike to the next. We assume that the membrane curves uniformly around the capsid, with a radius of curvature equal to the radius of the capsid. Since there are 80 binding sites, the bending energy, E, required to advance from one site to the next will be that needed to curve the membrane around 1/80th of the total surface area of the capsid:

$$E = \frac{B}{2} \oint\!\!\oint C^2 \, dA = \frac{8\pi B}{80} \qquad (1)$$

where B is the bending modulus of the lipid bilayer and C is the mean curvature (6, 7). Using a value of $B = 2 \times 10^{-12}$ ergs (6, 8, 9, 11, 18) we find a bending energy of 15 $k_B T$, where k_B is Boltzmann's constant and T the absolute temperature. Because the membrane may not be flaccid, additional energy arising from surface tractions is required to deform the membrane not attached to the capsid (see Fig. 1). Measurements of cell surface tensions vary between 0.035

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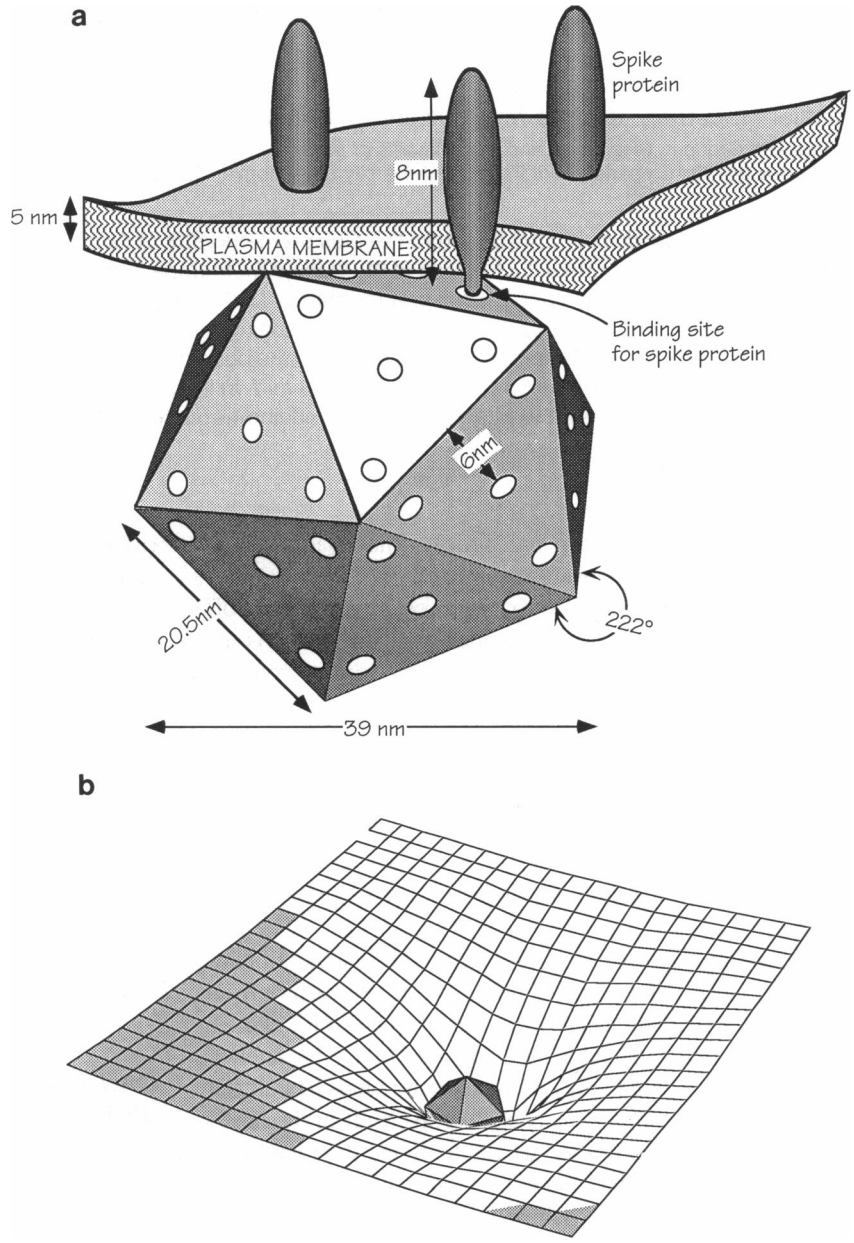

FIGURE 1 (*a*) The capsid is a rough icosohedron of diameter 39 nm. On each face are four binding sites for spike proteins, each of which are about 8 nm long and span the 5-nm membrane. (*b*) The computed virus position in the midst of the budding process. Both drawings are roughly to scale.

and 0.039 dyne/cm (8–10), which yields an additional bending resistance of 10–30 k_BT. The calculation leading to this figure is given in Appendix A.

We can estimate the time scale for this process using the asymptotic form of the mean first passage time, $\bar{T}$, for a particle in a harmonic well with diffusion coefficient D to reach a potential energy $E \gg kT$ (11):

$$\bar{T} = \frac{x^2}{2D}\left[\frac{1}{\sqrt{2}}\left(\frac{E}{kT}\right)^{-3/2} e^{E/kT}\right]. \qquad (2)$$

We assume that the drag on the membrane is equivalent to that of a disc of radius $x \approx 10^{-7}$ cm, the distance between binding sites, and a diffusion constant in cytoplasm of $D \approx (kT/16\eta a) \approx 8.3 \times 10^{-8}$ cm^2/s.

Fig. 2 *a* shows a plot of Eq. 2 for these parameters. We see that, if the bending modulus is greater than about 3×10^{-13} ergs, budding will take longer than the experimental upper limit of 20 min. Because of the exponential dependence on energy, this time will increase dramatically for bending moduli in the range typical of lipid bilayers, $B \approx 10^{-12}$ ergs.

Squeezing water from between the capsid and the membrane slows the wrapping process

As the membrane wraps the capsid it must squeeze water out from between the membrane and the capsid, which will slow down the wrapping process. We approximate the physics of a membrane moving toward a wall by replacing it with a disc of radius b, where $2b$ is the distance between sites. The dif-

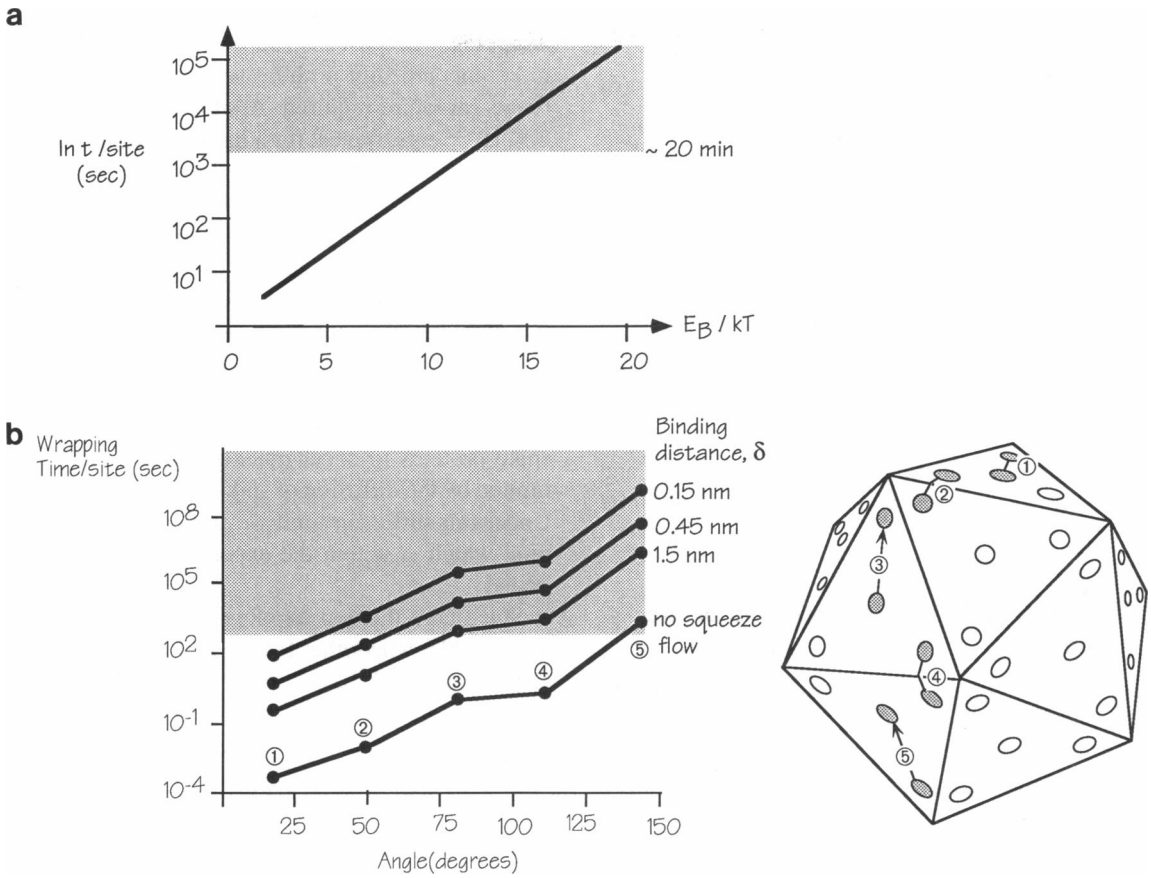

FIGURE 2 The computed wrapping time as a function of (*a*) bending energy, and (*b*) wrapping angle for several adjacent binding sites, and for various values of the binding distance. The computed points correspond to the adjacent sites shown in the inset diagram of the virus capsid. The shaded region indicates wrapping times larger than the observed maximum of 20 min.

fusion coefficient of this disc is given by

$$D(x) = \frac{D_0}{1 + (b/x)^3}, \tag{3}$$

where $D_o = k_B T/16\eta b$, and x is the distance from the wall (8). The probability of finding the diffusing particle at a distance x from the wall, $P(x,t)$, satisfies the modified diffusion equation

$$\frac{\partial P}{\partial t} = -\frac{\partial}{\partial x}\left(-D(x)\frac{\partial P}{\partial x} + \frac{f(x)}{16\eta b}P\right) \tag{4}$$

where $f(x)$ is the elastic force. We will assume that the disk sticks at a distance δ from the capsid, which represents the length of the cytoplasmic tail of the spike protein. By solving the corresponding backward (adjoint) equation, we find that the mean first passage time for sticking has the asymptotic form (Appendix B) as follows.

$$\bar{T} \sim \sqrt{\pi}\frac{(\bar{x} - \delta)^2}{4D_0}\left(\frac{E}{k_B T}\right)^{-3/2}\exp\left(\frac{E}{k_B T}\right)\left[1 + \left(\frac{b}{\delta}\right)^3\right] \tag{5}$$

The dependence of $\bar{T}$ on wrapping angle is shown in Fig. 2 for various values of δ, with and without squeeze flow. For the middle value of δ, wrapping would require nearly a year.

Summing the effects of bending elasticity and hydrodynamic resistance gives the wrapping time as a function of energy and of wrapping angle. Fig. 2 shows that the wrapping time increases so rapidly with wrapping angle and membrane bending stiffness that it is unlikely that thermal fluctuations could drive capsid budding unless the membrane bending modulus were significantly reduced.

How fast can the membrane wrap if it is limited by the availability of spike proteins?

In the above calculation we assumed that the membrane bound to a waiting spike protein as soon as it came within the binding distance. However, in order for a sufficiently large fluctuation to advance wrapping a spike protein must be available for binding to the capsid. Therefore, we compute the wrapping rate assuming that the availability of spike proteins is the rate-limiting step. Let $\rho(x,t)$ be the density of spike proteins in the membrane. Then $\rho(x,t)$

satisfies the diffusion equation

$$\frac{\partial \rho}{\partial t} = D\nabla^2\rho + S_o \tag{6}$$

where the source term, S_o, is the number of glycoprotein spikes introduced into the cell surface per unit area per second (this assumes that the spikes are introduced randomly over the surface). Quinn et al. (12) have measured the production of glycoprotein spikes in an infected BHK cell, which they find to be 1.2×10^5 molecules/cell/min. They found the average area of the plasma membrane in BHK cells to be 3400 μm^2, and the concentration of virions is greater than $10/\mu m^2$ of plasma membrane. Thus $S = (1.2 \times 10^5)/3400 = 35.5$ molecules/min/μm^2, and $a^2 = 1/10$ capsids/$\mu m^2 = 0.1$ μm^2/capsid. From this we can compute lower and upper bounds of 11 and 22 min for the budding time (Appendix C).

Viruses bud from highly curved regions of the plasma membrane

A curious feature of virus budding is that buds frequently aggregate on cellular projections and other regions of high curvature (13). One possible explanation for this is the phenomenon of curvature-induced membrane instability. The extracellular portion of most viral spike proteins is much larger than its cytoplasmic domain. Therefore, upon insertion into the bilayer a spike imparts an asymmetrical bending moment which will tend to curve the membrane outwards, forming a local "bump." The bending energy associated with this bump is given by Eq. 1 if we replace the mean curvature, C, by $(C - C_o)$, where C_o is the intrinsic curvature due to the protein. A computation shows that the total bending energy for a collection of isolated bumps is higher than if the bumps were aggregated into one large bump. Moreover, a simple model demonstrates that a uniform distribution of such bumps is dynamically unstable, leading to an aggregation (Appendix D). The reason is easy to see. A spike protein in a planar membrane diffuses isotropically. In a region of increasing curvature, however, there is a gradient in the splay of the lipid chains, and so the entropic motions of the lipid chains exert an asymmetric pressure on the protein. Thus the diffusive motion of the spike proteins has a curvature bias equivalent to a convective term of the form $J_c = (\kappa\nabla C)S$, where S is the density of spikes, and κ is proportional to the membrane thickness and the protein diffusion constant. This is sufficient to ensure that a uniform distribution of spikes is dynamically unstable, and that the spikes will tend to accumulate in regions of high curvature.

DISCUSSION

We have calculated the budding time for the thermal trapping model of virus budding. We find that the energy required to deform the membrane sufficiently to bring a spike protein to the next available binding site is between 10 and 30 kT, depending on location of the binding site on the capsid. As wrapping proceeds, each subsequent binding event becomes more energetically prohibitive due to the increasing membrane curvature required. Assuming a bending modulus of 2×10^{-12} ergs, typical for a lipid bilayer, the budding would take longer than the experimental upper limit of 20 min. In addition, as the membrane wraps around the capsid water must be removed from between the membrane and the capsid. This slows the rate of wrapping significantly. Taken together, this would appear to exclude the possibility of thermal fluctuations driving budding. However, if the bending modulus is less than 3×10^{-13} erg, which is outside the range of most experimental measurements, the required membrane fluctuations would be frequent enough for binding of the spike proteins to occur quickly, even if the binding rate is limited by the diffusion of spike proteins to the binding sites. Under this diffusion limit we find a budding time of 10–20 min, which is within the experimentally determined upper bound.

Since pure thermal trapping of membrane fluctuations is not feasible, what other forces could deform the membrane? Oster et al. (2, 14) have suggested a model in which the spike proteins act as a surfactant, lowering the interfacial tension of the outer leaflet. This upsets the balance of interfacial tensions between the two leaflets of the bilayer with two results: (i) a small bending moment is generated across the bilayer that slightly bows the membrane outwards, (ii) the membrane's net bending modulus is reduced. As the spike proteins diffuse to and bind the capsid, their combined effect wraps the membrane around the capsid. In support of this notion, recent studies show that a difference in thermal expansivity between the inner and outer leaflets can drive budding in lipid vesicles (7, 15). The source of the spike proteins' surfactant activity may arise in several ways. For example, positive charges near the membrane interface will attract counterions to the interfacial region, and the counterion pressure opposes the local interfacial tension, resulting in a local lowering of the outer leaflet's interfacial tension. In this connection, Garry et al. (16) have shown that budding is inhibited in BHK-S and CE cells incubated in low NaCl medium. Alternatively, the spike protein might act in the same fashion as the peptides cardiotoxin and melittin, which associate with specific lipid types to produce a local bending moment (17). Finally, some unspecified conformational change in the spike or capsid proteins could drive the membrane deformation. However, in the absence of an evidence for this we prefer the simpler, physical explanation.

APPENDICES

A. How much energy does it take to wrap the capsid?

In order to compute the effect of membrane tension on wrapping energy we treat a section of the membrane as a semi-infinite strip of width equal to the distance between binding sites on the capsid $\approx 10^{-6}$ cm. The inclination of the membrane has initial value ψ_0, determined by the geometry of the capsid,

and asymptotically approaches 0. We compute the angle $\psi(s)$ by minimizing the bending energy

$$E = h \int \left[\frac{B}{2} \left(\frac{\partial \Psi}{\partial s} \right)^2 + \sigma \right] ds \tag{A1}$$

where σ is the membrane tension. Subtracting the energies from adjacent sites gives the energy required to wrap one site:

$$\Delta E(\Psi_0) = 8h\sqrt{\sigma B}\left(\sin^2 \frac{\Psi_0'}{2} - \sin^2 \frac{\Psi_0}{2} \right) - h\sigma R(\sin \Psi_0' - \sin \Psi_0)$$
$$+ 2\pi Rh\,|\Delta\Psi_0| \left(\sigma + \frac{B}{2R^2} \right) \tag{A2}$$

where ψ_0 and ψ_0' are the initial inclinations of the membrane at adjacent sites. This additional bending energy is 11–50 kT, depending on what value of h we choose; i.e., either the distance between nodes on the capsid or the persistence length of the membrane, $\lambda \sim 5 \times 10^{-6}$ cm. This calculation underestimates the energy in that it only includes one of two principal curvatures. We have also calculated the change in energy numerically, by discretizing the three-dimensional form of the above integral.

$$E = \sum_i \left(\frac{1}{2} BC_i^2 + \sigma \right) \sqrt{g_i} \Delta\theta \Delta z \tag{A3}$$

where the mean curvature, C_i, and determinant of the metric tensor, g_i, are calculated at each grid point, i. Using an optimization algorithm, the coordinates $r_i(\theta_i, z_i)$ are varied to minimize the integral, with three nodes fixed at the capsid surface and two nodes flat at large distances from the capsid. The icosahedral sites are located by projecting them onto a sphere. We obtain energies between 10 and 30 kT to advance the membrane from one site on the icosahedron to the next, depending on the pair of binding sites considered. Fig. 2 *a* shows how wrapping time varies with bending energy.

B. The effect of squeeze flow on wrapping time

Here we calculate how much the squeeze flow affects the time it takes for the fluctuating membrane to advance one binding site. Since the site-to-site displacement is small we can approximate the elasticity of the membrane by a quadratic potential. The squeeze flow is equivalent to a variable viscous drag coefficient, $\zeta \sim 1 + (b/x)^3$ (8). The membrane binds when it comes within a distance δ of the wall equal to the distance the spike protein protrudes from the surface of the membrane. We compute the site-to-site wrapping time by calculating the first passage time for the disc-shaped piece of membrane to hit the absorbing boundary. This is an underestimate since it assumes that irreversible binding occurs at the very first encounter.

We compute the first passage time from the Fokker-Planck equation for the probability density $P(x, t)$:

$$\frac{\partial P}{\partial x} = \frac{1}{2} \frac{\partial^2}{\partial x^2} [m_2(x)P] + \frac{\partial}{\partial x}[-m_1(x)P]$$
$$= -\frac{\partial}{\partial x} \left[-D(x) \frac{\partial P}{\partial x} + \frac{f(x)}{\zeta(x)} P \right] \tag{B1}$$

where $D(x) = m_2(x)/2$, $m_1(x) = -\partial D/\partial x + f(x)/\zeta$, the elastic force is $f(x) = -\kappa(x - \bar{x})$ and the drag coefficient is $\zeta(x) = (k_B T/D(x)) = 16\eta b[1 + (b/x)^3]$. At equilibrium, the flux vanishes and so the density, $\bar{P}$, must satisfy

$$\frac{f(x)}{\zeta(x)} \bar{P} = D(x) \frac{d\bar{P}}{dt} \tag{B2}$$

i.e.,

$$\bar{P} \sim \exp\left[-\frac{(x - \bar{x})^2}{2\sigma^2} \right] \tag{B3}$$

where $\sigma^2 = k_B T/\kappa$. This is equivalent to the identity $\frac{1}{2}(d/dx)\,(m_2\bar{P}) = m_1\bar{P}$, i.e., $\bar{P}' = f \times P/k_B T$.

Starting from position $x = X$, the mean first passage time to reach $x = \delta$, denoted $T(\delta \mid X)$, is given by the adjoint equation to Eq. B1:

$$\frac{1}{2} m_2(X) \frac{\partial^2 T}{\partial X^2} + m_1(x) \frac{\partial T}{\partial X} = -1 \tag{B4}$$

where m_1 and m_2 are as before. The boundary conditions are (i) $x = \delta$ is absorbing:

$$T(X = \delta) = 0 \tag{B5}$$

and infinity is reflecting (19):

$$\lim_{L \to \infty} \left. \frac{\partial T(X)}{\partial X} \right|_{X=L} = 0. \tag{B6}$$

Multiplying both sides of Eq. B4 by $\bar{P}$ and using the above identity yields the following.

$$-\bar{P}(X) = \frac{1}{2} m_2(X)\bar{P}(X) \frac{\partial T^2(\delta \mid X)}{\partial x^2} + m_1(X)\bar{P}(X) \frac{\partial T(\delta \mid X)}{\partial x}$$
$$= \frac{\partial}{\partial X} \left[\frac{1}{2} m_2(X)\bar{P}(X) \frac{dT(\delta \mid X)}{dX} \right] \tag{B7}$$

Integrating this twice produces

$$T(X) = \int_\delta^\infty \frac{C_1 - \int_\delta^z \bar{P}(\xi)\, d\xi}{\frac{1}{2} m_2(z)\bar{P}(z)} dz + C_2. \tag{B8}$$

The absorbing boundary condition (Eq. B5) requires that $C_2 = 0$, and the reflecting boundary condition (Eq. B6) gives $C_1 = \int_\delta^L \bar{P}(\xi) d\xi$. Thus the formula for the first passage time starting from X is

$$T(X) = 2 \int_\delta^X \frac{\int_z^\infty \bar{P}(\xi)\, d\xi}{m_2(z)\bar{P}(z)} dz \tag{B9}$$

The mean first passage time is obtained by integrating over all possible initial conditions. We approximate the mean first passage time as the time to go from X, the center of the harmonic well—the most likely position—to δ, the sticking point:

$$\bar{T}(X) = 2 \int_\delta^X \frac{1/2\sqrt{2\pi\sigma^2}\left[1 + \mathrm{erf}\left(\frac{z - X}{\sigma\sqrt{2}} \right) \right]}{m_2(z)\bar{P}(z)} dz$$
$$= \int_\delta^X \frac{\sqrt{\frac{\pi k_B T}{2\kappa}}\left[1 + \mathrm{erf}\left(\frac{z - X}{\sqrt{2k_B T/\kappa}} \right) \right]}{D(z)\bar{P}(z)} dz \tag{B10}$$

where the error function $\mathrm{erf}(x) = (2/\sqrt{\pi}) \int_0^x \exp(-\xi^2) d\xi$. Eq. B10 must be integrated numerically; however, an approximate expression is obtained as follows. Inserting the expression for the variable diffusion coefficient we obtain

$$T(\bar{X}) = \frac{1}{2D_0} \sqrt{2\pi\sigma^2} \int_\delta^X \left[1 + \mathrm{erf}\left(\frac{z - X}{\sigma\sqrt{2}} \right) \right] \times \left[1 + \frac{b^3}{z^3} \right]$$
$$\times \exp\left[\frac{(z - X)^2}{2\sigma^2} \right] dz \sim \frac{1}{2D_0} \sqrt{2\pi\sigma^2} \int_\delta^X \left[1 + \frac{b^3}{z^3} \right]$$
$$\times \exp\left[\frac{(z - X)^2}{2\sigma^2} \right] dz. \tag{B11}$$

Letting $s = 1 - (z - X)^2/(X - \delta)^2$ and substituting for z, the integral becomes

$$T(X) = \frac{1}{4D_0}\sqrt{2\pi\sigma^2}\exp\left[\frac{(X-\delta)^2}{2\sigma^2}\right](X-\delta) \times \int_0^1 \left\{1 + \frac{b^3}{[X-(X-\delta)\sqrt{1-s}]^3}\right\} \times \exp\left[\frac{-(X-\delta)^2 S}{2\sigma^2}\right]\frac{1}{\sqrt{1-s}}\,ds$$

$$= \sqrt{\pi}\frac{(X-\delta)^2}{4D_0}\frac{1}{\sqrt{\Delta E'}}\exp(\Delta E') \times I \qquad \text{(B12)}$$

where $\Delta E' = (X-\delta)^2/2\sigma^2$, $u = \sqrt{\Delta E'}$, and

$$I \sim \int_0^1 \left\{\left[1 + \left(\frac{b}{\delta}\right)^3\right] + \frac{s}{2}\left[1 + \left(\frac{b}{\delta}\right)^3\left[4 - 3\left(\frac{X}{\delta}\right)\right]\right] \ldots\right\}\exp(-u^2 s)\,ds$$

$$\sim \frac{1}{u^2}\left(\left[1 + \left(\frac{b}{\delta}\right)^3\right] + \frac{1}{2u^2}\left[1 + (b/\delta)^3[4 - 3(X/\delta)]\right] + \ldots\right). \qquad \text{(B13)}$$

Thus an approximate expression for the mean first passage time starting from $x = X$ is as follows.

$$T(X) = \sqrt{\pi}\frac{(X-\delta)^2}{4D_0}\frac{1}{(\Delta E')^{3/2}}e^{\Delta E'}\left(\left[1 + \left(\frac{b}{\delta}\right)^3\right] + \frac{1}{2\Delta E'}\left\{1 + \left(\frac{b}{\delta}\right)^3\left[4 - 3\left(\frac{X}{\delta}\right)\right]\right\} + \ldots\right) \qquad \text{(B14)}$$

Reversible binding

So far we have considered the binding of the spike to the capsid as irreversible and instantaneous: the capsid sticks instantly to the spike the first time it encounters it. We can lift this restriction by making the boundary condition at $x = \delta$ partially reflecting

$$-D\frac{dT(X)}{dX} + \kappa T(X)\bigg|_{x=\delta} = 0. \qquad \text{(B15)}$$

Note that this is equivalent to the forward equation boundary condition:

$$-D\frac{dC}{dx} + \frac{fD}{kT}C = -\kappa C. \qquad \text{(B16)}$$

The constant κ has the interpretation

$$\kappa = \frac{\text{Prob(absorb)}}{\text{Prob(reflect)}} \times k_D \qquad \text{(B17)}$$

where $k_D = 2D/L$ is the diffusion-limited binding constant on an interval of length L (20). With this boundary condition the constant C_2 in Eq. B8 is not zero, but is given by the following.

$$C_2 = \frac{\int_\delta^\infty \bar{P}(y)\,dy}{\kappa\bar{P}(\delta)} \approx \sqrt{\pi}\frac{X-\delta}{\kappa\sqrt{\Delta E'}}e^{\Delta E'} \qquad \text{(B18)}$$

This yields

$$T(\delta) = T_0 + \sqrt{\pi}\frac{X-\delta}{\kappa\sqrt{\Delta E'}}e^{\Delta E'} \qquad \text{(B19)}$$

where T_0 is the first passage time with an absorbing barrier.

C. Diffusion limited wrapping

We model the cell surface as a regular lattice of nucleocapsids with glycoprotein spikes that can diffuse laterally in the plane of the membrane. The distance between two capsids in the same row or column equals a where $1/a^2$ is the number of capsids per unit area of membrane. By symmetry, this problem is equivalent to a single virus in a square of length a with reflecting boundary conditions. We can bound the rate of absorption of spike proteins by the capsid by considering two concentric circles around the square boundary and solving the analogous reflecting boundary problem for each. Since we are looking at diffusion-limited kinetics, we assume that, whenever a spike protein diffuses in to the capsid, it binds almost instantly. Assuming steady state and imposing the reflecting boundary condition at $r = a$, the number of spikes sticking to the capsid/second is

$$2\pi RD\frac{\partial\rho}{\partial r}\bigg|_{r=R} = 2\pi R\left(\frac{Sa^2}{2R} - \frac{SR}{2}\right) \approx \pi a^2 S. \qquad \text{(C1)}$$

For the outer circle of radius $a\sqrt{2}$, the rate of absorption equals $2\pi Sa^2$. Thus $S = (1.2 \times 10^5)/3400 = 35.5$ molecules/min/μm^2, and $a^2 = 1/10$ capsids/μm^2 = 0.1 μm^2/capsid. So the lower rate of glycoprotein sticking is $\pi \times 35.3 \times 0.1 = 11$ spikes/min, and the upper rate is 22. Dividing the 240 spikes by the number of spikes per min, we get 11 and 22 min for the lower and upper bound on the budding time.

Petersen et al. (21) have calculated the fraction of mobile spike proteins from photobleaching recovery experiments and find it to be less than 0.1. Averaging the density of spike proteins in the membrane from our diffusion calculation, we can express the mobile fraction as the ratio of this density to the density of spike proteins bound to nucleocapsids. Assuming that the average capsid has 120 spike proteins bound to it, the density of bound spike proteins is then 120 times the density of capsids. From this we estimate a mobile spike protein fraction of 0.001. Thus it is not necessary to assume, as Petersen has done, that budding begins in carrier vesicles en route to the cell surface. Rather, the assumption that budding takes place at the cell surface is sufficient to explain the low mobile fraction.

D. Spikes collect in regions of high membrane curvature

The following one-dimensional calculation illustrates the principle of curvature-induced aggregation. The displacement of a cylindrical sheet (e.g., a filopod), $u(x,t)$, with bending modulus B obeys

$$\zeta\frac{\partial u}{\partial t} = \frac{\partial^2}{\partial X^2}(B(C - C_0 S) - \tau u) \qquad \text{(D1)}$$

where $C \approx (\partial^2 u/\partial x^2)$ is the (approximate) curvature, $S(x,t)$ is the density of spike proteins, ζ is a frictional coefficient, B the bending modulus, C_0 the curvature (or bending couple) imparted/spike protein, and τ the membrane tension. The spike proteins diffuse according to

$$\frac{\partial S}{\partial t} = -\frac{\partial}{\partial x}\left[-D\frac{\partial S}{\partial x} + \left(\kappa\frac{\partial C}{\partial x}\right)S\right] \qquad \text{(D2)}$$

where D is the diffusion coefficient of the spikes in the membrane. The second term is the flux of spikes toward regions of higher curvature (i.e., the campylotactic flux, from the Greek *campylos* = curvature). The coefficient, κ depends on membrane thickness and protein diffusion coefficient; therefore, the effect of curvature will only be important in a certain range of membrane properties. A linear stability analysis of these equations shows that the uniform distribution, $\bar{S}$ = constant, is unstable against perturbations larger than a critical wavenumber. A more detailed analysis of curvature-induced aggregation was given previously by Leibler (22, 23).

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