Chapter 13

Free Energy Minimization for Vesicle Translocation Through a Narrow Pore

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Abstract

This chapter presents a mathematical formulation for the translocation process of a vesicle through a narrow pore. The effect of the deformation of the vesicle while passing through the pore causes a penalty in the free energy, while the existence of an external driving force assists. We formulate the free energy landscape of the vesicle in terms of bending and stretching energy and use Fokker-Plank formalism to calculate the first-passage translocation time. We also address various modifications that can be done to this approach to make it work for different systems.

Key words Translocation, Vesicle surface energy, Fokker-Plank mechanism, Helfrich free energy

1 Introduction

The control of vesicles' translocation through pores is fundamental to drug delivery and transdermal applications [1–3]. Although the exact process varies based on chemical and physical details of the system, it can be studied using a coarse-grained theoretical model [4]. The geometric properties of vesicles as manifested by the curvature and surface tension of the membrane fluctuate due to the interaction with the pore and other environmental obstacles. Analyzing the statistical mechanical properties of these macroscopic features gives us a significant insight into the behavior of these complex systems. In order to study the passage of a vesicle through a pore, we adopted the method which was originally introduced by Deserno and Gelbart [5]. In their model, they minimized the energy of the vesicle's adhesion, membrane tension, and curvature, under fixed volume constraint, to describe the phenomenon of endocytosis.

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2 Models

For this chapter, we assume the readers already have some knowledge in mathematics and geometry. We consider the vesicle to be initially a sphere in the form of r_0, and assume that it is incompressible and only the area of the vesicle changes during the translocation thorough the pore, which has the shape of a cylinder with radius d ($d < r_0$) and length L.

2.1 Translocation Process Model Assumption

We model the vesicle to undergo smooth deformations while passing through the pore. The schematics process of the whole passage of the translocation of the vesicle from the donor to the receiver compartment is shown in Fig. 1. When the vesicle reaches the pore entrance (Fig. 1a), its shape starts to undergo deformation in order to fill up the pore (Fig. 1b). The vesicle moves further into the pore, until it completely fills up the pore and enters the receiving compartment (Fig. 1c). The process continues until the vesicle has completely left the donor compartment, but has still filled the pore partially (Fig. 1d). Finally, the vesicle completely leaves the pore and reaches to a new relaxed state on the receiving compartment (Fig. 1e).

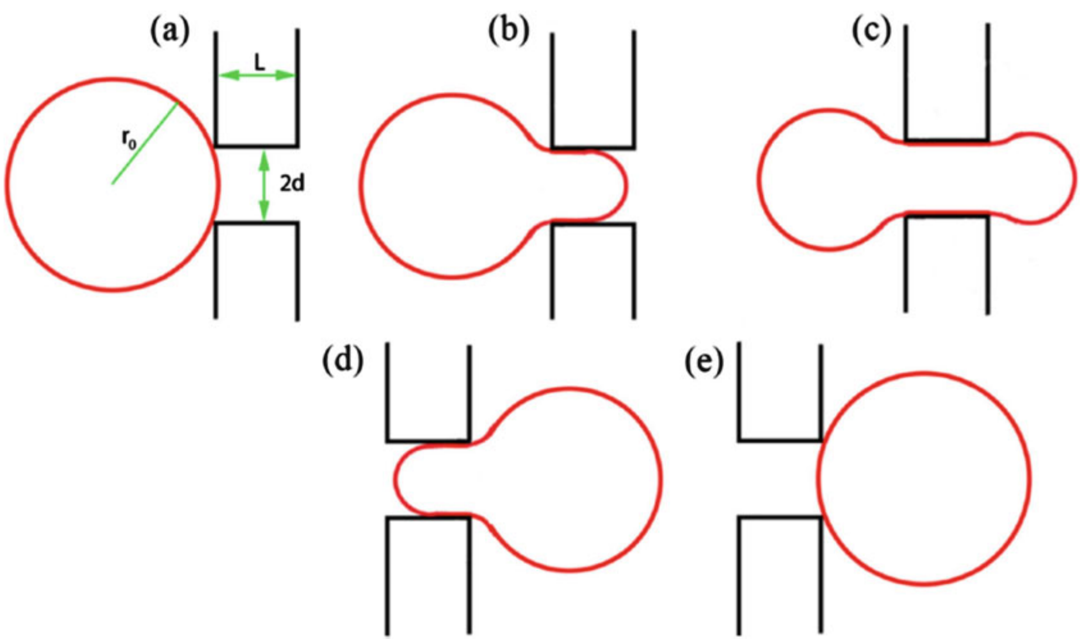

Fig. 1 Schematics of the translocation of a spherical vesicle of initial radius r_0 through a narrow pore of radius d and length L. (**a**) Initial state of the vesicle in the donor compartment, (**b**) partial penetration of the vesicle into the pore, (**c**) filling of the pore with the remainder of the vesicle partitioned into both the donor and receiver compartments, (**d**) partial filling of the pore in the exit stage, and (**e**) final state of the vesicle in the receiver compartment

2.2 Helfrich Free Energy

The free energy landscape of the vesicle due to the fluctuation on its shape from the free vesicle is in the form of Helfrich free energy:

$$F_{\mathrm{H}} = \frac{1}{2}\,\kappa_{\mathrm{c}} \oint dA (2\mathrm{H} - \mathrm{c}_0)^2 + \frac{1}{2\mathrm{A}_0}\lambda\,(\mathrm{A} - \mathrm{A}_0)^2. \qquad (1)$$

The first term in Eq. 1 comes from bending the surface, while the second term accounts for the stretching of the surface. κ_c and λ are, respectively, bending modulus and stretching modulus. The bending term in the free energy is the integral of $(2H - c_0)^2$ over the whole surface. Here, $H = \frac{1}{2}\left(\frac{1}{r_1} + \frac{1}{r_2}\right)$ is the mean curvature, with $r_{1,\,2}$ being the principal radii at any given point on the surface. The spontaneous curvature c_0 comes from the molecular structure of the surface. In general, there is an energy cost to bend a surface, which is given by the integral of the quadratic difference between mean curvature and spontaneous curvature over the whole surface of the vesicle. Helfrich free energy originally included the Gaussian curvature, which after integration gives a topological invariant. However, its contribution to the free energy can be ignored since in our model we do not take into consideration the topological changes in the vesicle.

The second term in the free energy comes from the change on the surface area from when it is relaxed.

2.3 Filling Stage

The stage at which the vesicle starts penetrating the pore but has not entered the receiving compartment is called the filling stage. During this stage the deformed shape of the vesicle is shown in Fig. 2a. To avoid edge penalty on the surface, the curvature should be finite and smooth at all points over the surface, including the point at which the vesicle meets the pore entrance. This can be obtained by introducing a hypothetical torus at the contact point

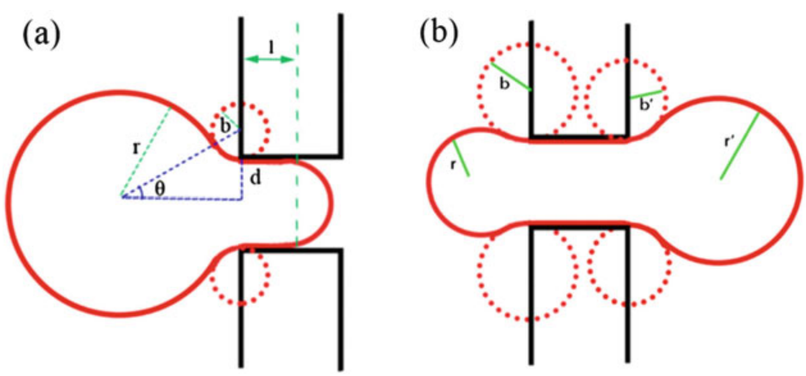

Fig. 2 Various parameters used to define the free energy of the deformed vesicle. (**a**) Filling stage: *r* is the radius of the partial sphere, *b* is the small radius of the toroidal vesicle. Dotted lines show the cross section of a hypothetical torus that keeps the curvature of the vesicle smooth. θ is the angle between the center of the spherical part and the center of the small toroidal ring. (**b**) Crossing stage: Two toroids are needed. b', r', and θ' correspond to the toroid on the receiver side, and *b*, *r*, and θ correspond to the donor side as in part (**a**)

between the pore and the vesicle. This torus has the inner radius of b and outer radius of $b + d$, with b being an unknown parameter. The dashed-line circle in Fig. 2a shows the cross-section of this torus. To calculate the volume of the vesicle in the donor side we add the volume of the partial sphere to the volume of the cone that fits into the empty space of the partial sphere. This cone has sharp edges that extend beyond the boundary of the completed sphere; therefore, you then have to subtract the volume of the partial toroidal section. The total form of the vesicle can be divided into multiple parts: a partial sphere with radius r and cutting angle θ, a partial torus with inner and outer radius b and $b + d$ and angle θ, a cylinder with radius d and length $l\,(l < L)$, and a hemisphere with radius d. The geometrical parameters of the partial shapes are as follows.

2.3.1 Partial Sphere

The volume of a sphere with radius r and a missing conical angle θ is

$$V_{\text{sphere}} = \frac{2}{3}\pi(1 + \cos\theta)r^3, \tag{2}$$

and the surface of this partial sphere is given by

$$A_{\text{sphere}} = 2\pi(1 + \cos\theta)r^2. \tag{3}$$

The mean radius of curvature on the surface of the sphere is constant and is equal to r.

2.3.2 Partial Torus

The volume of a partial torus with inner and outer radii b and $b + d$, between the angles $\nu = \frac{\pi}{2} + \theta$ and π, as it is shown in Fig. 2a, is

$$V_{\text{torus}} = \pi b^2\left[(b + d)\left(\frac{\pi}{2} - \theta\right) - \frac{2}{3}b\cos\theta\right], \tag{4a}$$

and the volume of a cone with angle θ and radius of $b + d$ is

$$V_{\text{cone}} = \frac{1}{3}\pi(b + d)^3\cot\theta. \tag{4b}$$

The surface area is given by

$$A_{\text{torus}} = 2\pi b\left[(b + d)\left(\frac{\pi}{2} - \theta\right) - b\cos\theta\right]. \tag{5}$$

The mean radius of curvature of the surface of the torus at angle ν is

$$H = \frac{b + d + 2b\cos\nu}{2b(b + d + b\cos\nu)}, \tag{6}$$

where b and θ are related to each other with geometrical confinement:

$$\sin\theta = \frac{b + d}{r + b}, \tag{7a}$$

$$b = \frac{r \sin\theta - d}{1 - \sin\theta}. \tag{7b}$$

The total volume of the vesicle in the donor compartment will be equal to $V_{\text{sphere}} + V_{\text{cone}} - V_{\text{torus}}$.

2.3.3 Cylinder

The volume of the cylinder with length l is

$$V_{\text{cylinder}} = \pi d^2 l. \tag{8}$$

The surface area is given by

$$A_{\text{cylinder}} = 2\pi dl. \tag{9}$$

The mean radius of curvature on the surface of the sphere is constant and is equal to $\frac{1}{2d}$.

2.3.4 Hemisphere

The volume of a hemisphere with radius d is

$$V_{\text{hemisphere}} = \frac{2}{3}\pi d^3. \tag{10}$$

The surface area is given by

$$A_{\text{hemisphere}} = 2\pi d^2. \tag{11}$$

Now that we have set up different geometrical parameters of various possible shapes of the vesicle in the filling stage, we can start reviewing crossing stage.

2.4 Crossing Stage

The crossing stage starts when the vesicle enters the receiving compartment, and it lasts until the vesicle leaves the donor compartment. In this stage, the vesicle occupies both the donor and receiving compartments, as well as the pore completely. The form of the vesicle for this stage can be defined in a similar manner as the filling stage. The general form of the vesicle can be divided into a partial sphere and a partial toroid for either donor or receiving compartments with different radii and angles on each side and a cylinder with the form of the pore and the length of L. A schematic figure of this stage is depicted in Fig. 2b. In this stage, in addition to b, r, and θ in the donor compartment, we have b', r', and θ' for the receiving compartment. Similar to Eqs. 7a and 7b, b', r', and θ' are related to each other by the following formulas:

$$\sin\theta' = \frac{b' + d}{r' + b'}, \tag{12a}$$

$$b' = \frac{r' \sin\theta' - d}{1 - \sin\theta'}. \tag{12b}$$

2.5 Depletion Stage

The final stage, at which the vesicle has completely left the donor compartment but is filling the pore partially is called the depletion stage. The geometrical structure of the vesicle is the same as the filling stage, but it happens in the receiving compartment.

2.6 Volume Constraint

For most biological systems and for bilayer membranes, the vesicle is incompressible. However, one can add the compressibility parameter to the system. The initial volume of a spherical vesicle with radius r_0 is

$$V_0 = \frac{4}{3}\pi r_0^3. \tag{13}$$

In order to parametrize the translocation process, we need to define the translocation coordinate, α, which is the ratio of the volume of the vesicle that passed the pore entrance at any given time (V_{passed}) to the initial volume of the vesicle during the translocation process:

$$\alpha = \frac{V_{\text{passed}}}{V_0}. \tag{14}$$

If we define the volume ratio of the pore to the vesicle as β

$$\beta = \frac{V_{\text{pore}}}{V_0} = \frac{\pi d^2 L}{V_0}, \tag{15}$$

then $\alpha = 0$ is the starting time and $\alpha = 1 + \beta$ represents the end of the translocation process.

2.7 External Force

Translocation only happens if there is an external force pushing the vesicle in the donor compartment toward the receiving compartment through the pore. The most common form of such a force for vesicles is osmotic pressure. We can consider that an external pressure P_1 is applied to the vesicle with volume V_0 in the donor compartment, which is higher than the pressure in the receiver compartment P_2. If we assume that this pressure difference between donor and receiver compartments is constant, then the external contribution to the free energy will be

$$F_{\text{ext}} = -\Delta P V = -\Delta P V_0 \frac{V_{\text{passed}}}{V_0}. \tag{16}$$

Using Eq. 14 and defining an energy term

$$f_0 = \Delta P V_0 (1 + \beta),$$

we can now write the external contribution to the free energy in Eq. 16 as a function of the translocation coordinate

$$F_{\text{ext}} = -f_0 \frac{\alpha}{1 + \beta}. \tag{17}$$

In the process of translocation α varies between 0 and $1 + \beta$. However, when the vesicle is completely out of the donor compartment, $\alpha = 1$. And from this time on we take $F_{\text{ext}} = -f_0\left(1/_{1+\beta}\right)$ for the rest of the process.

2.8 Fokker-Plank Formalism

The kinetics of the translocation process can be studied using the free energy landscape. Using the translocation coordinate, α, and the Fokker-Planck formalism, we can calculate the average translocation time

$$\tau = \frac{1}{\kappa} \int_0^{1+\beta} d\alpha_1 \int_0^{\alpha_1} d\alpha_2 \mathrm{e}^{[F(\alpha_1)-F(\alpha_2)]/k_\mathrm{B}T}, \tag{18}$$

with

$$F(\alpha) = F_\mathrm{H} + F_{\text{ext}}, \tag{19}$$

which are defined in Eqs. 1 and 17. In order to apply Fokker-Planck formalism we need to assume that the translocation time is longer than the relaxation time of thevesicle, which is the time required by the vesicle to get back to its equilibrium form after any distortion in its shape. This will then satisfy the detailed balance requirement of Fokker-Planck formalism. As vesicle goes through the pore, there will be a local friction, and it is incorporated in formula 18 by parameter κ. This parameter is also set as the unit of time for our problem.

3 Methods

After knowing all the details, we can calculate the free energy landscape as a function of the translocation coordinate α. Before the translocation process starts, the bending and the stretching terms in the Helfrich free energy are

$$F_{0,\text{bend}} = \frac{\kappa_\mathrm{c}}{2}\left[4\pi r_0^2\left(\frac{2}{r_0} - c_0\right)^2\right] = \kappa_\mathrm{c}(2\pi)(2 - r_0 c_0)^2, \tag{20a}$$

$$F_{0,\text{stretch}} = 0. \tag{20b}$$

The bending and stretching energy contributions to the free energy on each stage of translocation process are calculated in the following sections.

3.1 Filling Stage

In the filling stage $0 < \alpha < \beta$. The volume constraint in Eq. 13 can be written as

$$V_{\text{donor}} = (1-\alpha)V_0 = V_{\text{sphere}} + V_{\text{cone}} - V_{\text{torus}}. \tag{21}$$

The rest of the vesicle can be used to get the length of the cylinder, which is filled

$$\pi d^2 l + \frac{2}{3}\pi d^3 = \alpha V_0 = \frac{4}{3}\pi\alpha r_0^3,$$

$$l = \frac{2}{3} d \left(2\alpha \frac{r_0^3}{d^3} - 1 \right). \tag{22}$$

Using Eqs. 2, 4a and 4b and substituting b from Eq. 7b, we obtain $r(\theta)$ for any given value of α. Both r and θ should be real. Also, as it can be seen in Fig. 2a, r and θ are bounded parameters:

$$d < r < r_0, \tag{23a}$$

$$\sin^{-1} \frac{d}{r_0} < \theta < \frac{\pi}{2}. \tag{23b}$$

Also, since part of the vesicle can practically pass the pore entrance before touching the edge of the pore, we have a mathematical limit for the minimum value of α:

$$\alpha_{\min} = \frac{1}{2} \frac{d^3}{r_0^3} < \alpha. \tag{23c}$$

The bending free energy is

$$F_{\mathrm{I,bend}} = F_{\mathrm{I,b,sphere}} + F_{\mathrm{I,b,torus}} + F_{\mathrm{I,b,cylinder}} + F_{\mathrm{I,b,hemisphere}}. \tag{24}$$

The first term is

$$\begin{aligned} F_{\mathrm{I,b,sphere}} &= \frac{1}{2} \kappa_c \int dA \left(\frac{2}{r} - c_0 \right)^2 \\ &= \frac{1}{2} \kappa_c \, (2\pi)(2 - r c_0)^2 \, (1 + \cos\theta). \end{aligned} \tag{25}$$

The second term is

$$F_{\mathrm{I,b,torus}} = \frac{1}{2} \kappa_c \int dA \left(\frac{b + d + 2\, b \cos\nu}{2b(b + d + b\cos\nu)} - c_0 \right)^2,$$

$$\begin{aligned} F_{\mathrm{I,b,torus}} = \frac{1}{2}\kappa_c \Bigg\{ & -2\pi(c_0 b + 2)^2 \cos\ \theta + 2\pi c_0 (c_0 b + 2)\left(\frac{\pi}{2} - \theta\right)(b + d) \\ & + \frac{2\pi (b+d)^2}{b\sqrt{d}\sqrt{2b + d}} \left[\pi - 2\tan^{-1}\left(\frac{\sqrt{d}}{\sqrt{2b+d}} \tan\left(\frac{\theta}{2} + \frac{\pi}{4} \right) \right) \right] \Bigg\}. \end{aligned} \tag{26}$$

The last two terms in Eq. 24 are

$$\begin{aligned} F_{\mathrm{I,b,cylinder}} &= \frac{1}{2} \kappa_c \pi d^2 l \left(\frac{1}{d} - c_0 \right)^2 \\ &= \frac{1}{2} \kappa_c \left(\frac{2}{3} \pi \right) \left(2\alpha \frac{r_0^3}{d^3} - 1 \right) (1 - c_0 d)^2, \end{aligned} \tag{27}$$

$$F_{\mathrm{I,b,hemisphere}} = \frac{1}{2} \kappa_c \int dA \left(\frac{2}{d} - c_0 \right)^2 = \frac{1}{2} \kappa_c (2\pi)(2 - c_0 d)^2. \tag{28}$$

The stretching term is

$$F_{\mathrm{I,stretch}} = \frac{1}{2A_0}\lambda(A_{\mathrm{I}} - A_0)^2 = \frac{1}{2A_0}\lambda(\Delta A_{\mathrm{I}})^2, \tag{29}$$

with

$$\Delta A_{\mathrm{I}} = A_{\mathrm{sphere}} + A_{\mathrm{torus}} + A_{\mathrm{cylinder}} + A_{\mathrm{hemisphere}} - 4\pi r_0^2. \tag{30}$$

Each term in Eq. 29 is already calculated in the materials section. For any given α, after substituting $r(\theta)$ of the volume constraint, the free energy will be only a function of r (or θ). Now that we have the free energy as a function of α, we can minimize the free energy equation with respect to θ and get the free energy landscape at any α.

3.2 Crossing Stage

In the crossing stage, $\beta < \alpha < 1$. We introduce the counterpart parameters, which corresponds to the received compartment with prime.

$$V_{\mathrm{donor}} = (1-\alpha)V_0 = V_{\mathrm{sphere}} + V_{\mathrm{cone}} - V_{\mathrm{torus}}, \tag{31}$$

$$V_{\mathrm{receiver}} = (1-\alpha')V_0 = V'_{\mathrm{sphere}} + V'_{\mathrm{cone}} - V'_{\mathrm{torus}}, \tag{32}$$

with $(1 - \alpha) + (1 - \alpha') + \beta = 1$, or $\alpha' = 1 + \beta - \alpha$. The bending energy for this stage is

$$F_{\mathrm{II,bend}} = F_{\mathrm{II,b,sphere}} + F_{\mathrm{II,b,torus}} + F_{\mathrm{II,b,pore}} + F'_{\mathrm{II,b,sphere}} + F'_{\mathrm{II,b,torus}}. \tag{33}$$

With assistance of Eqs. 12a and 12b, each term in Eq. 33 is as follows

$$F_{\mathrm{II,b,sphere}} = \frac{1}{2}\kappa_{\mathrm{c}}\,(2\pi)(2 - rc_0)^2\,(1 + \cos\theta), \tag{34a}$$

$$F'_{\mathrm{II,b,sphere}} = \frac{1}{2}\kappa_{\mathrm{c}}\,(2\pi)(2 - r'c_0)^2\,(1 + \cos\ \theta'), \tag{34b}$$

$$F_{\mathrm{II,b,torus}} = \frac{1}{2}\kappa_{\mathrm{c}}\Bigg\{-2\pi(c_0b+2)^2\cos\theta + 2\pi c_0(c_0b+2)\left(\frac{\pi}{2}-\theta\right)(b+d) + \frac{2\pi(b+d)^2}{b\sqrt{d}\sqrt{2b+d}}\left[\pi - 2\tan^{-1}\left(\frac{\sqrt{d}}{\sqrt{2b+d}}\tan\left(\frac{\theta}{2}+\frac{\pi}{4}\right)\right)\right]\Bigg\}, \tag{34c}$$

$$F_{\mathrm{II,b,torus}} = \frac{1}{2}\kappa_{\mathrm{c}}\Bigg\{-2\pi(c_0b'+2)^2\cos\ \theta' + 2\pi c_0(c_0b'+2)\left(\frac{\pi}{2}-\theta'\right)(b'+d) + \frac{2\pi(b'+d)^2}{b'\sqrt{d}\sqrt{2b'+d}}\left[\pi - 2\tan^{-1}\left(\frac{\sqrt{d}}{\sqrt{2b'+d}}\tan\left(\frac{\theta'}{2}+\frac{\pi}{4}\right)\right)\right]\Bigg\}, \tag{34d}$$

$$F_{\mathrm{II,b,pore}} = \frac{1}{2}\kappa_{\mathrm{c}}\pi d^2 L\left(\frac{1}{d} - c_0\right)^2 = \frac{1}{2}\kappa_{\mathrm{c}}\left(\frac{8}{3}\pi\beta\frac{r_0^3}{d^3}\right)\left(1 - c_0 d\right)^2. \tag{34e}$$

Where L is the length of the pore. The same as filling stage, the stretching energy ist

$$F_{\mathrm{II,stretch}} = \frac{1}{2A_0}\,\lambda\,(A_{\mathrm{II}} - A_0)^2 = \frac{1}{2A_0}\lambda(\Delta A_{\mathrm{II}})^2, \tag{35a}$$

with

$$\Delta A_{\mathrm{II}} = A_{\mathrm{sphere}} + A_{\mathrm{torus}} + A_{\mathrm{pore}} + A'_{\mathrm{sphere}} + A'_{\mathrm{torus}} - 4\pi r_0^2, \tag{35b}$$

with r, b, and θ in A, respectively, replaced by r', b', and θ' in A'. The minimization process is the same as the filling stage. Since r should monotonically and continuously reduce from r_0 to d, we can minimize the free energy separately for the donor and receiver parameters (r and r') to get the free energy landscape.

3.3 Depletion Stage

For $1 < \alpha < 1 + \beta$, we are in the depletion stage. In this stage, everything is completely the same as the filling stage, by replacing α with $\alpha' = 1 + \beta - \alpha$.

3.4 Free Energy Barrier

The general form of the free energy, as we calculated in previous sections, is shown in Fig. 3. Figure 3a shows the free energy landscape without an external driving force ($f_0 = 0$). Figure 3b shows the free energy landscape with the existence of an external driving force ($f_0 \neq 0$). While we assume the spontaneous curvature to be 0, there is no restriction on having a nonzero c_0.

3.5 Average Translocation Time

Having the free energy landscape, we can use Eq. 18 to find the first passage translocation time. In order to solve the double integral in Eq. 18, we need to increment α with a value δ. At each step we calculate the numerical value of the free energy and then sum over its numerical values. The results for an arbitrary value of κ_c and λ for multiple values of r_0 is shown in Fig. 4. For example, with the driving force $f_0 = 15$ and the radius of the vesicle $r_0 = 1.75$ with bending modulus $\kappa_c = 3$ and stretching modulus $\lambda = 5$, the translocation time will be equal to 500. Changing any parameter here has a big impact on the outcomes. In the following paragraph we give some examples.

3.5.1 Vesicle Radius r_0

Let us increase the radius of vesicle to twice its value (i.e., $r_0 = 2.5$). To achieve the same translocation as in previous example (i.e., $\tau = 500$) one needs to increase the external free energy drive f_0 by more than 4.6 times to $f_0 = 70$.

3.5.2 Elastic Moduli κ_c and λ

If all the parameters are the same except elastic modulus κ_c, then increasing it by a factor of 2 will increase the translocation time τ up to six orders of magnitude. The effect of λ is much weaker in comparison to κ_c.

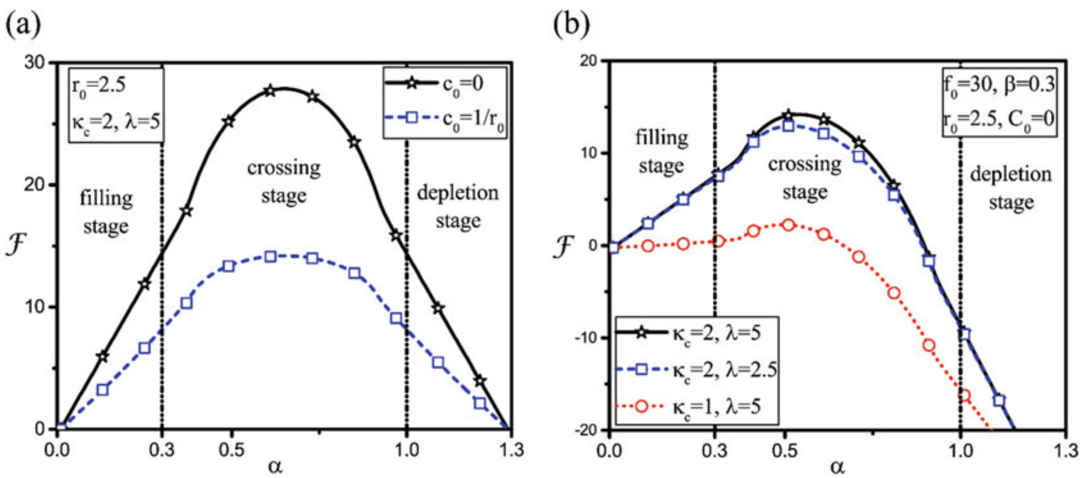

Fig. 3 The free energy barrier with and without an external driving force. (**a**) The Helfrich free energy landscape as a function of α. There is no external driving force ($f_0 = 0$). Three different stages of the vesicle translocation (filling, crossing, and depletion) is shown. The black line shows the curve when the spontaneous curvature is zero, and the blue dashed line is for the case when there is a nonzero ($c_0 \neq 0$). Here ($r_0 = 2.5d$, $\beta = 0.3$, $\kappa_c = 2$ and $\lambda = 5$). (**b**) The Helfrich free energy landscape as a function of α in the presence of an external driving force ($f_0 \neq 0$). While we assume the spontaneous curvature to be 0 ($c_0 = 0$), there is no restriction on having a nonzero c_0. We plotted the curve for different values of stretching and bending moduli. The black line shows the case when $\kappa_c = 2$ and $\lambda = 5$, the blue dashed line has a different value, $\lambda = 2.5$, and the red dashed line shows the curve when $\kappa_c = 1$ and $\lambda = 5$

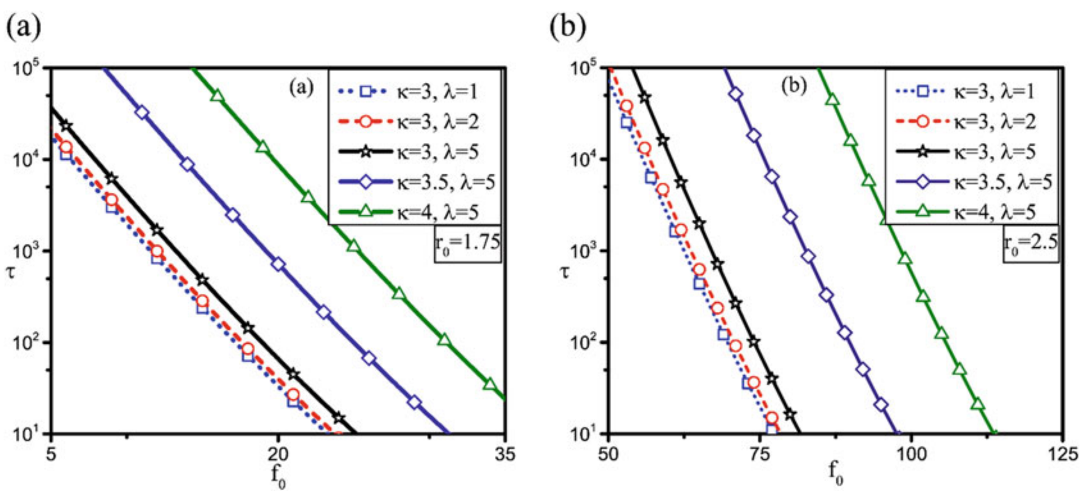

Fig. 4 The effect of the external driving force f_0 on translocation time τ. (**a**) Shows the case when the vesicle's radius is $r_0 = 1.75$ and in (**b**) $r_0 = 2.5$. The effect of different stretching and bending moduli is depicted in both (**a**) and (**b**)

4 Notes

1. In order to run the program, it is enough to have Python3 in the system. We use Numpy, Scipy, and matplotlib libraries to perform the calculations. The program has many comments within itself. It is designed to be user friendly and people from different backgrounds will be able to use it at any level they want. The part which is for the user is at the bottom of the program; there are possibilities for changing the parameters as the user wishes. There are a few notes that should be considered while using the program, which are mentioned in this section.

   VesicleTranslocation(r0, β, Fext, κ_c, λ) accepts r0, β, Fext, κ_c, and λ as input, and as output it prints the energies at each α and plots the figure which shows the relationship between free energy and α. The user also needs to enter the values for the κ_c and λ, which are bending modulus and a stretching modulus, respectively.

   Users can obtain the program from Github. In order to run the program, one needs first to go to the section which starts with "if __name__ == '__main__':". In this section the user can choose parameters in VesicleTranslocation(r0, β, Fext, κ_c, λ) as inputs to start the computation. If the user has an IDE for python, he/she can simply run the program in IDE. Otherwise from the terminal the following command will run the program:

   Python vesicletranslocation.py

   More information about the program is available at the Github address (https://github.com/hamid-shojaei/vesicle-translocation).
2. One needs to pay attention to the range of β. If the size of the vesicle is smaller than the pore, then $\beta \geq 1$ and the free energy will not change inside the pore. If the length of the pore is zero ($l = 0$), we do not have crossing stage, and it will be just filling and depletion. When there is an external force ($f_0 \neq 0$), the form of the external force will be different if it is in the depletion process.
3. One possible extension to this work is to consider a conical shape instead of a cylindrical geometry for the pore. The crossing stage formulas should be modified. In this case, Eqs. 4a, 8, 9, 24, and 33 will be modified. It would be interesting to see the impact of geometry of the pore on the properties of translocation since the real biological environment can be far from perfect.
4. In this work we ignored the electrostatic effects of vesicle-fluid interface on the translocation process. A more detailed and

comprehensive study which includes the effect of a charged vesicle is another direction for future works.

5. A different shape shape of the vesicle can be chosen, but then one needs to change H in Eq. 1 and all the equations in different stages of translocation, beside those which describes a cylindrical pore. Choosing a different vesicle shaple can also potentially impact the translocation process.

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