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Statistical mechanics of active vesicles and the size distribution paradox

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ABSTRACT

Vesicles are the primary modes of communication and transport in cell biology. Conventional wisdom based on thermodynamic equilibrium says that vesicles should have a certain minimum size and size distribution dictated by their thermal fluctuations. However, there is compelling experimental evidence that vesicles exhibit a vast variety of size distributions depending on their formation process and function which cannot be explained by equilibrium statistical mechanics alone. We investigate a non-equilibrium statistical mechanics-based model to understand the role of active membranes on the size distribution of vesicles. Active membranes contain proteins that use external energy sources, such as adenosine triphosphate hydrolysis, and are known to exert forces on the membrane during their activity to carry out different biological functions. The central idea behind our model is that activity, attributed to different sources, impacts vesicle fluctuations in two opposing ways — by active noise which enhances fluctuations, and membrane tension which decreases fluctuations. The interplay of active fluctuations and active tension endows the vesicles with the ability to achieve size distributions that are deemed improbable by equilibrium statistical mechanics. We show that our model for active vesicles, based on linearized curvature elasticity, can reproduce different experimental data for vesicle size distributions available in the literature by varying the activity. Elucidating how these vesicles achieve such diverse size distributions can open avenues for a deeper understanding of physiological and pathological processes and help design vesicles for diagnostics and drug delivery applications.

1. Introduction

Vesicles are key players in myriads of physiological processes that are essential for homeostasis, ranging from intracellular transport, and storage to intercellular signaling, trafficking, and communication (Alberts et al., 2002). Extracellular vesicles are now known to play important roles even in diseases such as heart disease, neurodegenerative diseases, and cancer (Kumar et al., 2024). Exosomes, a particular class of vesicles, are being studied for various clinical applications including therapeutic agents, vehicles for drug delivery and diagnostics, and tissue regeneration and repair mechanisms (Di Bella, 2022).

Vesicles are essentially small structures consisting of an outer membrane made of a lipid bilayer enclosing a fluid-filled volume containing cellular material such as proteins, lipids, nucleic acids like DNA and RNA, nutrients, and waste products (Alberts et al., 2002). The vesicular membrane often contains embedded proteins or protein complexes that are involved in different functions including transmembrane transport, membrane fusion, and vesicle stability. Depending on the function of the vesicles, they can vary significantly in size, ranging from a few nanometers to several micrometers. Studies on extracellular vesicles classify them based

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on size and functions with exosomes being the smallest vesicles ranging from 30–150 nm and microsomes ranging from 100 nm to a micron (Di Bella, 2022; Ginini et al., 2019; Ronquist, 2019). Significant research has focused on liposomes which are artificial vesicles that serve as models to study membrane properties, and processes found in real cells and are promising vehicles for targeted drug delivery applications. These synthetic vesicles span a wide size range from tens of nanometers for small unilamellar vesicles to micrometers for giant unilamellar vesicles with the preparation method influencing the final size distribution obtained (R Mozafari and Linder, 2011; Zhang and Sun, 2021).

This considerable variation in sizes of vesicles found in nature as well as synthesized in the laboratory begs the question: how do vesicles control their sizes based on their biological function? Numerous experimental studies in cell biology have been dedicated to addressing this question. Owing to the crucial role of vesicles, the field is vast, undoubtedly, and we only cite a few studies that inspired the proposed theoretical study (Coldren et al., 2003; Menger et al., 1989; Men et al., 2016; Korgel et al., 1998; Kanno et al., 2002; Xu et al., 2013). Using their method for the synthesis of polymersomes, a certain class of vesicles, Menger et al. (1989) found that the size distribution of small unilamellar vesicles was affected by the presence of calcium ions, pH, and ionic strength but remained insensitive to the sonication method and duration. In contrast, in a more recent study, Men et al. (2016) show that the size and distribution of the polymersomes are controlled by the solvent content and sonication time. Korgel et al. (1998) observe that unilamellar vesicles formed by extrusion can exhibit non-Gaussian size distributions and the average size is controlled by the pore size used for extrusion. A common observation in all these experimental results is that vesicle distribution has three important features – a certain lower cutoff vesicle size, a mean vesicle size, and the standard deviation of the bell-shaped curve.

The equilibrium size distribution of vesicles has also been studied theoretically over decades (Helfrich, 1986; Kleinert, 1986; Ahmadpoor and Sharma, 2016; Huang et al., 2017). Pioneering work by Helfrich (1986) proposed a method to estimate the probability distribution of vesicles based on equilibrium statistical mechanics taking into account the thermal fluctuations. Thirty years later, Ahmadpoor and Sharma (2016) enriched Helfrich's model with nonlinear curvature elasticity to demonstrate that for tensionless membranes, nonlinear curvature elasticity was important to capture the threshold size below which vesicles do not exist and attributed it to entropic effects. Indeed, Helfrich's model, based on linearized curvature elasticity, cannot capture this threshold or minimum vesicle size in the absence of surface tension and spontaneous curvature. In another recent study, Huang et al. (2017) used nonlinear curvature elasticity to study the dependence of membrane size, spontaneous curvature, and membrane stiffness on vesicle formation and size distribution. As expected for these theories based on thermal fluctuations, the only parameters that can change the vesicle size distribution are bending modulus of the membrane, the number of amphiphilic molecules comprising the vesicle, and ambient temperature which in turn affects the entropic interactions. Although these studies have provided unprecedented insights into vesicle size distribution, we find it rather perplexing that the vast variation in vesicle size distribution observed in nature cannot be explained solely on the basis of equilibrium statistical mechanics.

In recent years, there has been a growing consensus that biological membranes need not be "passive" entities exhibiting thermal fluctuations only but are rather "active" and harness energy from intrinsic or extrinsic energy sources to execute specific biophysical functions (Ramaswamy and Rao, 2001; Ramaswamy, 2010; Bowick et al., 2022; Lee et al., 2017). These active membranes contain special proteins that aid in the trafficking of molecules such as ions, lipids, or proteins, across the membranes as part of a variety of vital physiological processes. As these active proteins perform their functions, they exert forces on the membrane that could be generated by a chemical reaction such as actin polymerization, adenosine triphosphate hydrolysis, mechanical stresses, electric fields or directly from light (Turlier and Betz, 2018). Several theoretical studies have demonstrated that vesicles made of active membranes indeed exhibit enhanced fluctuations (Loubet et al., 2012; Iyer et al., 2023; Kulkarni, 2023). These observations present the enticing possibility that vesicular membranes may in fact be active and that activity plays a more pronounced role in governing the size distribution of vesicles. This is schematically represented by Fig. 1 which illustrates how probability distribution is related to fluctuations of vesicles and that different degrees of fluctuations can lead to vesicles of different sizes. In contrast to passive vesicles which can exist only within a certain size range, active vesicles can achieve smaller sizes by reducing the fluctuations and larger sizes by increasing the fluctuations, thus explaining the size distribution paradox.

Here, we present a continuum mechanics model for active vesicles to explain the aforementioned size distribution paradox. Since activity drives vesicles away from equilibrium, we first perform a dynamic analysis of fluctuating vesicles to derive their statistical mechanics results in the presence of active forces. The steady state fluctuation spectra is then used to estimate the effective free energy and the vesicle size distribution by taking into account thermal and active fluctuations. We work with the hypothesis that activity, attributed to different sources, impacts membrane fluctuations in two opposing ways — as active noise which enhances fluctuations, and as membrane tension which decreases fluctuations. Our model shows that the interplay of active noise and active tension can explain how active vesicles achieve size distributions that are deemed improbable for passive vesicles. To the best of our knowledge, this is the first model that can reproduce a wide variety of published experimental results.

2. Mechanics of active vesicles

Biological membranes and vesicles have been studied extensively using continuum mechanics and equilibrium statistical mechanics treatments (Lipowsky, 1991; Safran, 1994; Nelson et al., 2004; Steigmann, 2018; Seifert, 1999). Studies on equilibrium thermal fluctuations of soft matter have led to remarkable insights into a variety of problems such as entropic interactions between fluctuating membranes (Helfrich, 1978; Gompper and Kroll, 1989; Freund, 2012; Hanlumyuang et al., 2014; Grasinger and Sharma, 2024), effect of edges (Biria et al., 2013; Zelisko et al., 2017), pore formation in membranes (Farago and Santangelo, 2005), membrane inclusions and interactions (Santangelo and Farago, 2007; Carotenuto et al., 2020; Liao and Purohit, 2021), stability of membranes (Zhong-can and Helfrich, 1989; Givli et al., 2012; Deseri et al., 2016), electromechanical coupling (Liu and Sharma,

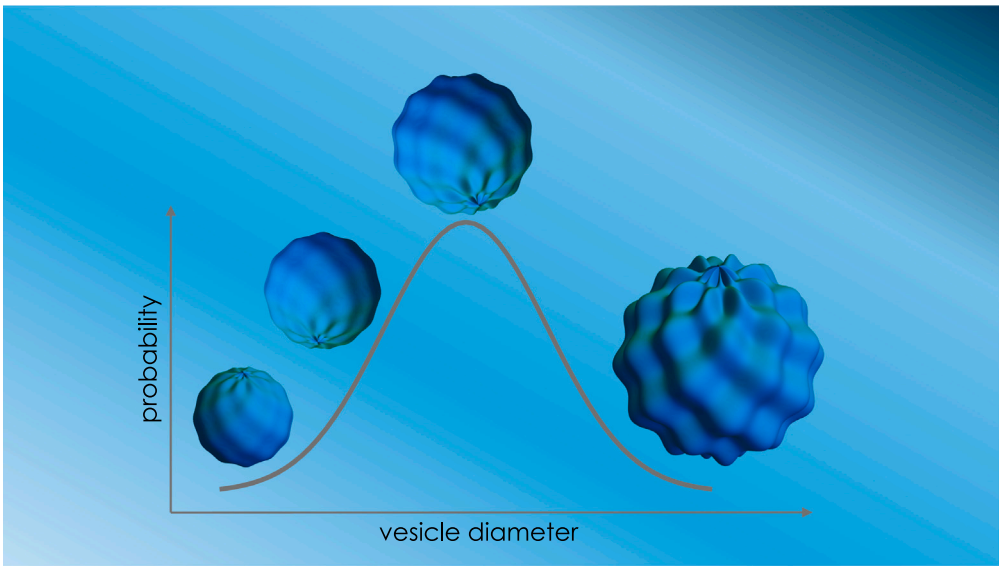

Fig. 1. Schematic showing the probability distribution of fluctuating vesicles of different sizes. Contrary to the vast variation in vesicles sizes found in nature, equilibrium statistical mechanics dictates that vesicles should exist within a certain size range based on their thermal fluctuations. We demonstrate that active vesicles may access their own energy source to circumvent equilibrium considerations and achieve desired size distributions by modulating their fluctuations, thus explaining the size distribution paradox.

2013; Nguyen et al., 2013; Grasinger et al., 2021; Khandagale et al., 2024), active filaments (Liao et al., 2020), and statistical mechanics of vesicles (Helfrich, 1986; Milner and Safran, 1987; Kleinert, 1986; Schneider et al., 1984; Seifert, 1999; Lomholt, 2006) among many others.

Below, we summarize the general formulation for deriving the equations of motion for passive vesicles to build context and then present our theory for active vesicles. For a vesicle in static equilibrium (in the absence of thermal fluctuations) the minimization of the potential energy yields the classical shape equation (Biria et al., 2013). For a vesicle in thermal equilibrium, the Hamiltonian is used to derive the probability distribution function or the equipartition theorem is used directly to yield the fluctuation spectra for the vesicle (Helfrich, 1986). In contrast, an active vesicle is not in thermal equilibrium, by definition, and hence requires a dynamic analysis of the fluctuating vesicle to account for the viscous dissipation due to the embedding fluid. This is achieved by a hydrodynamics treatment of the fluid surrounding the vesicle (Seifert, 1999; Arroyo and DeSimone, 2009).

2.1. Passive vesicles

From a mechanics perspective, membranes are considered elastic sheets that are resistant to in-plane deformations but can bend easily due to out-of-plane deformations (Phillips et al., 1998). Let $\mathbb{S}$ be the surface of a fluctuating quasi-spherical vesicle with mean radius r_0, defined as $\mathbb{S} := \{\mathbf{R} \in \mathbb{R}^3 : |\mathbf{R}| = r_0\}$. Consider a small perturbation of the surface $\mathbb{S}$ to $\mathbb{S}_\varepsilon$ which takes a point on $\mathbb{S}$ with position vector $\mathbf{R}$ to $\mathbf{R}_\varepsilon$,

$$\mathbf{R}_\varepsilon = \mathbf{R} + \varepsilon \mathbf{u} \tag{1}$$

with

$$\mathbf{u} = \mathbf{u}_t + U\mathbf{n} = u_\alpha \mathbf{a}_\alpha + U\mathbf{n}, \quad \alpha = 1, 2 \tag{2}$$

where $\mathbf{n}$ is a unit normal field on surface $\mathbb{S}$, $U(\theta, \phi)$ is the normal variation and u_α are the tangential variations along directions $\mathbf{a}_\alpha$ in the tangent plane. The total potential energy, $\mathcal{F}$, is expressed as (Biria et al., 2013)

$$\mathcal{F} = \int_{\mathbb{S}} (\psi + \sigma)\, d\mathbb{S}, \tag{3}$$

where ψ is the elastic energy density, σ is the so-called surface tension, and $\mathbb{S}$ is the surface of the fluctuating membrane. Mathematically, σ is the Lagrange multiplier associated with the energy penalty for areal changes due to deformation (Safran, 1994; Nelson et al., 2004; Steigmann, 2018; Phillips et al., 1998). Linearized curvature elasticity describes the potential energy $\psi = \bar{\psi}(H, K)$ by the celebrated Helfrich–Canham–Evans Hamiltonian (Canham, 1970; Helfrich, 1973; Evans, 1974) as

$$\bar{\psi}(H, K) = \frac{1}{2}\kappa(2H - H_0)^2 + \bar{\kappa}(K - K_0), \tag{4}$$

where κ and $\bar{\kappa}$ are the bending moduli that represent the energetic costs associated with changes in the mean curvature H, and the Gaussian curvature K, respectively. H_0 and K_0 denote the corresponding spontaneous curvatures. In linearized curvature elasticity, assuming no topological changes and spontaneous curvatures, the potential energy of the membrane reduces to

$$\bar{\psi}(H) = \frac{1}{2}\kappa(2H)^2. \tag{5}$$

Then, the variational derivative of the potential energy with respect to U is derived as (Biria et al., 2013)

$$\mathcal{F}_U = 2\kappa\Delta_S H - 2H\,\sigma \tag{6}$$

where Δ_S is the surface Laplacian (Kulkarni, 2023; Biria et al., 2013). Normalizing U with respect to the mean radius r_0 by defining a dimensionless variable $u(\theta,\phi) = U(\theta,\phi)/r_0$, we can expand H in terms of u as follows (Helfrich, 1986),

$$H = \frac{1}{r_0}\left[-1 + u + \frac{1}{2}\underline{\Delta}u - u^2 - u\underline{\Delta}u\right] \tag{7}$$

while keeping terms only up to quadratic in u. Here, $\underline{\Delta}$ is the normalized surface Laplacian with the following expression in spherical coordinates,

$$\underline{\Delta} = \frac{1}{\sin\theta}\frac{\partial}{\partial\theta}\left(\sin\theta\frac{\partial}{\partial\theta}\right) + \frac{1}{\sin^2\theta}\frac{\partial^2}{\partial\phi^2} \tag{8}$$

The equations of motion for a fluctuating vesicle in dynamic equilibrium with the ambient fluid have been derived before and the details can be found in many works (Cai and Lubensky, 1994; Seifert, 1999; Arroyo and DeSimone, 2009; Kulkarni, 2023). The central idea is to solve the Stokes equation for the embedding fluid and establish force balance at the interface of the fluid and the vesicle membrane. We report the resulting governing equation here:

$$\frac{\eta}{\Gamma_\ell}\dot{u}_{\ell,m}(t) = -\frac{\kappa}{r_0^3}E_\ell\,u_{\ell,m} + \xi^{th}_{\ell,m}(t)\,, \tag{9}$$

where

$$E_\ell = (\ell+2)(\ell-1)\left[\ell(\ell+1) + \bar{\sigma}\right], \tag{10}$$

and

$$\Gamma_\ell = \frac{\ell(\ell+1)}{4\ell^3 + 6\ell^2 - 1}\,. \tag{11}$$

Eq. (9) is the classical over-damped Langevin equation in spherical coordinates for a quasi-spherical vesicle of mean radius r_0. $\bar{\sigma} = \sigma r_0^2/\kappa$ is the dimensionless surface tension and η is the viscosity of the embedding fluid. The normal displacement of the vesicle membrane is expressed in terms of spherical harmonics as

$$u(\theta,\phi) = \sum_{\ell\geq 0}^{\ell_{max}}\sum_{m=-\ell}^{\ell} u_{\ell,m}Y_{\ell,m}(\theta,\phi) \tag{12}$$

where $u_{\ell,-m} = u^*_{\ell,m}$ since $u(\theta,\phi)$ is real; $Y_{\ell,m}$ are separable in terms of Legendre polynomials, $P^m_\ell(\theta)$, as

$$Y_{\ell,m}(\theta,\phi) = P^m_\ell(\theta)e^{im\phi}\,, \tag{13}$$

and satisfy the eigenvalue equation,

$$\underline{\Delta}Y_{\ell,m} = -\ell(\ell+1)Y_{\ell,m}\,. \tag{14}$$

Since there are no topological changes in the problem of vesicle size distribution, the Hamiltonian in Eq. (5) is assumed to depend only on the mean curvature here. $\xi^{th}_{\ell,m}(t)$ denoting the thermal noise is uncorrelated in space and time and satisfies the fluctuation–dissipation theorem,

$$\langle\xi^{th}_{\ell,m}(t)\rangle = 0\,, \tag{15}$$

$$\langle\xi^{th}_{\ell,m}(t)\xi^{th}_{\ell',m'}(t')^*\rangle = 2\eta\frac{k_BT}{r_0^3}\frac{1}{\Gamma_\ell}\delta_{\ell\ell'}\delta_{mm'}\delta(t-t')\,, \tag{16}$$

Here, k_B is the Boltzmann constant and T is the ambient temperature. δ_{ij} is the Kronecker delta and $\delta(t)$ is the Dirac delta function. For vesicles exhibiting thermal fluctuations only, the dynamical analysis yields the same result for the fluctuation spectra as obtained from equilibrium statistical mechanics (Helfrich, 1986; Seifert, 1999; Kulkarni, 2023):

$$\langle|u_{\ell m}|^2\rangle = \frac{k_BT}{\kappa E_\ell}\,. \tag{17}$$

2.2. Active vesicles

The framework outlined above has been extended to active membranes and vesicles in prior studies. In Section 2.2.1, we summarize some of these studies which incorporate activity through a noise term. In Section 2.2.2, we propose an additional (and distinct) contribution through membrane tension. Section 2.2.3 presents the equations of motion for active vesicles under the influence of both active noise and active tension.

2.2.1. Active noise

Prior studies have argued that active membrane proteins exert random forces on the membrane during their activity and thereby lead to non-thermal fluctuations in addition to thermal fluctuations (Prost and Bruinsma, 1996; Ramaswamy et al., 2000; Loubet et al., 2012). These enhanced fluctuations have indeed been observed in experiments and considered signatures of activity (Turlier and Betz, 2018). Several studies have incorporated this activity by appending Eq. (9) with an active noise, denoted by $\xi^a_{\ell,m}(t)$. Based on experimental evidence, the active noise is assumed to be uncorrelated in space but has an exponentially decaying correlation in time (Loubet et al., 2012),

$$\langle \xi^a_{\ell,m}(t) \rangle = 0\,, \tag{18}$$

$$\langle \xi^a_{\ell,m}(0) \xi^a_{\ell,m}(t)^* \rangle = \chi_a x_\ell\, \delta_{\ell\ell'} \delta_{mm'} \exp\left[-\frac{|t|}{\tau_a}\right], \tag{19}$$

where χ_a is the amplitude of the active noise, and τ_a is the characteristic correlation time of the active process. x_ℓ is a function of the wave number based on the nature of the activity. By spherical symmetry, it is a function of ℓ only. It is important to emphasize that active noise, when appended to Eq. (9) only increases the fluctuations. As will become evident in the next section where we describe the vesicle size distribution model, larger fluctuations yield larger vesicle sizes (Kulkarni, 2023). Thus, we argue that active noise alone does not explain the diverse size distributions found in nature.

2.2.2. Active tension

In this work, we propose that active forces may modify the membrane fluctuations in a distinct way (than noise) which is incorporated through the membrane tension. To motivate this idea, we make two observations. First, consider a given vesicle fluctuating about a mean radius r_0. It is well-known that there will be a certain excess area that will determine how much the membrane can fluctuate. This areal constraint is used to determine the surface tension of the membrane, the Lagrange multiplier in Eq. (3) (Helfrich, 1986; Seifert, 1999). Thus, physically, the higher the surface tension, the lesser the (taut) membrane can fluctuate. Studies (Loubet et al., 2012; Girard et al., 2005) have shown that activity enhances the excess area of the membrane and in turn reduces the surface tension. This has been demonstrated by showing that active noise has a contribution to the surface tension through the areal constraint (Kulkarni, 2023). In other words, activity from a given source appears in the fluctuation spectra of a vesicle directly through the noise and indirectly by modifying the surface tension. However, our calculations reveal that for a given amplitude of the noise, the contribution to the fluctuations through the surface tension is much smaller than the contribution through noise directly. Hence, simply adding active noise only increases the fluctuations. Second, there is an emerging viewpoint in experimental studies that cells can indeed control their membrane tension by various means such as active forces applied by the cytoskeleton (Roffay et al., 2021; Maitra et al., 2014) and transmembrane flux of solutes (Saric and Freeman, 2021). Another recent study reported that changes in cholesterol can impact the membrane tension in vesicles (Biswas et al., 2019). Inspired by these observations, we propose that activity, attributed to different sources, impacts fluctuations through noise and tension separately.

2.2.3. Model for active vesicles

To incorporate the contributions from active noise and active tension separately in the theoretical framework for vesicles, we first append Eq. (3) with an active tension, denoted by σ_a,

$$\mathcal{F} = \int_{\mathbb{S}} (\psi + \sigma + \sigma_a)\, d\mathbb{S}\,. \tag{20}$$

The introduction of this new tension does not impact the derivation of the governing equation but modifies the coefficient given in Eq. (10) as

$$E^a_\ell = (\ell+2)(\ell-1)\left[\ell(\ell+1) + \bar{\sigma} + \bar{\sigma}_a\right], \tag{21}$$

with superscript a in E^a_ℓ denoting "active" and $\bar{\sigma}_a = \sigma_a r_0^2/\kappa$ being the non-dimensional active tension. Then, the over-damped Langevin equation for an active vesicle can be written as

$$\frac{\eta}{\Gamma_\ell}\dot{u}_{\ell,m}(t) = -\frac{\kappa}{r_0^3} E^a_\ell\, u_{\ell,m} + \xi^{th}_{\ell,m}(t) + \xi^a_{\ell,m}(t)\,. \tag{22}$$

Assuming steady state, from Eqs. (22) and (19), the fluctuation spectra for a fluctuating active vesicle is derived as (Kulkarni, 2023)

$$\langle |u_{\ell m}|^2 \rangle = \frac{k_B T}{\kappa E^a_\ell}\left(1 + \tilde{\chi}_a \frac{x_\ell}{E^a_\ell}\right), \tag{23}$$

where

$$\tilde{\chi}_a = \chi_a \frac{r_0^6}{k_B T \kappa}\,, \tag{24}$$

Eqs. (21)–(23) are the key results of this work. They summarize the governing equations and the statistical mechanical results based on the dynamical analysis of a quasi-spherical vesicle with active noise and active tension.

3. Size distribution of active vesicles

In this section, we apply our statistical mechanics results derived in Section 2 to study vesicle size distribution. We first present Helfrich's theory for estimating size distribution of vesicles in equilibrium. This theory relates the probability distribution for vesicles to the fluctuation spectra the vesicles through an effective free energy that takes into account the entropic effects due to fluctuations. In order to derive an explicit expression for this free energy and hence the probability distribution, we have to use the expressions for the fluctuation spectra derived in Section 2 for passive and active vesicles, that is, Eqs. (17) and (23) respectively.

According to Helfrich (1986), the probability distribution for a vesicle consisting of N amphiphilic molecules in equilibrium with free energy F is estimated as

$$w(N) = A\exp(-F/k_BT) \tag{25}$$

where A is determined by normalizing the probability distribution function using $\int_0^\infty w(N)\,dN = 1$. The free energy of a non-fluctuating spherical vesicle of radius r_0 is $F = \mu N + 8\pi\kappa$. The first term is the chemical contribution to the energy assuming that the vesicle is made of N amphiphilic molecules and the chemical potential is μ. The second term is the contribution from bending energy with mean curvature $H = 1/r_0$ and the total surface area $4\pi r_0^2$. To account for entropic effects, Helfrich proposed an effective free energy for a fluctuating vesicle by replacing the actual bending modulus with the effective bending modulus that takes into account the fluctuations:

$$F = \mu N + 8\pi\kappa_{\text{eff}}\,, \tag{26}$$

where κ_{eff} is the bending modulus renormalized due to fluctuations of the membrane. For tensionless membranes with linearized curvature elasticity, F is obtained as (Helfrich, 1986; Kleinert, 1986)

$$F = \mu N + 8\pi\left(\kappa - \frac{k_BT}{16\pi}\log N\right), \tag{27}$$

where N is estimated through the expression $N = \frac{4\pi r_0^2}{d^2}$ with d being the thickness of the membrane. The above well-known result shows that thermal fluctuations lead to a softening effect by reducing the apparent bending modulus.

Although active processes drive a vesicle away from equilibrium, here we consider the active vesicle to be in steady-state and close to equilibrium. Recent studies have considered non-equilibrium effects in active membranes in a similar manner by renormalizing the temperature which is an equilibrium concept (Turlier and Betz, 2019). This near-equilibrium assumption allows us to use the method proposed by Helfrich to estimate the size distribution of active vesicles by deriving an effective free energy which incorporates the effects of fluctuations due to activity in addition to thermal fluctuations.

We follow Ahmadpoor and Sharma (2016) to estimate this effective free energy from fluctuations. To this end, the additional free energy $\tilde{F}$ due to fluctuations of a spherical vesicle can be estimated from the change in apparent bending modulus as

$$\tilde{F} = \frac{k_BT}{2}\sum_{\ell,m}\log G_{\ell m} = 8\pi(\kappa - \kappa_{\text{eff}})\,, \tag{28}$$

where $G_{\ell m}$ is obtained from the fluctuation spectra as

$$\langle|u_{\ell m}|^2\rangle \approx \frac{k_BT}{2G_{\ell m}}\,. \tag{29}$$

Comparing Eqs. (23) and (29) yields

$$G_{\ell m} = \kappa\frac{E^a_\ell}{1+\tilde{f}_\ell}, \quad \text{where} \quad \tilde{f}_\ell = \frac{\tilde{\chi}_a x_\ell}{E^a_\ell}\,. \tag{30}$$

E^a_ℓ can be written in terms of the different modes of the spherical harmonics as

$$\kappa E^a_\ell = \kappa\left[\ell^2(\ell+1)^2 - 2\ell(\ell+1)\right] + \kappa(\bar{\sigma}+\bar{\sigma}_a)(\ell+2)(\ell-1) = c_1q^4 - c_2q^2 + c_3q^2 \tag{31}$$

where we have defined $q^2 = \ell(\ell+1)$ and approximated $(\ell+2)(\ell-1) \approx q^2$, so that

$$c_1 = \kappa,\;\; c_2 = \kappa,\;\; c_3 = \kappa(\sigma+\bar{\sigma}_a) \tag{32}$$

Substituting these expressions in Eq. (28), the expression for κ_{eff} is derived as

$$\kappa_{eff} = \kappa - \frac{k_bT}{16\pi}\left(\log N - (\bar{\sigma}+\bar{\sigma}_a)\log N + \sum_{l,m}\frac{\tilde{\chi}_a x_l}{E^a_\ell}\right). \tag{33}$$

Substituting this in Eq. (26) yields the free energy for active vesicles as

$$F = \mu N + 8\pi\kappa - \frac{k_bT}{2}\left(\log N - (\bar{\sigma}+\bar{\sigma}_a)\log N + \sum_{l,m}\frac{\tilde{\chi}_a x_l}{E^a_\ell}\right). \tag{34}$$

By substituting this in Eq. (25), we get the size distribution for vesicles with active noise and active tension. As we shall see in Section 4, this contribution resolves the size distribution paradox. Note that taking $\bar{\sigma}$, $\bar{\sigma}_a$, and $\tilde{\chi}_a$ to be zero recovers Helfrich's results for passive vesicles (Eq. (27)).

To evaluate the summation term in Eq. (34), we must discuss the expression for x_ℓ which depends on the nature of activity. The origin of active noise has been attributed to two types of forces in literature — curvature force, where the active proteins induce spontaneous curvature in the membrane and direct force, where the active proteins directly exert forces on the membrane. Explicit expressions for the free energy, and vesicle size distribution for the two cases are provided below.

Case I: Active noise due to curvature force

The curvature force is exerted by transmembrane proteins and ion pumps by affecting the spontaneous curvature and is described using $x_\ell = (\ell+2)^2(\ell-1)^2/4$ (Loubet et al., 2012). Isolating the summation term in (33), we have

$$\sum_{l,m} \frac{\tilde{\chi}_a x_l}{E^a_\ell} = \sum_{l,m} \kappa \frac{\tilde{\chi}_a(\ell+2)^2(\ell-1)^2}{4\,q^4(\kappa - \frac{\kappa}{q^2} + \frac{\kappa(\bar{\sigma}+\bar{\sigma}_a)}{q^2})} \tag{35}$$

$$\approx \sum_{l,m} \kappa \frac{\tilde{\chi}_a q^4}{4\,q^4\kappa} \tag{36}$$

$$= \sum_{l,m} \frac{\tilde{\chi}_a}{4} \tag{37}$$

$$= \frac{\tilde{\chi}_a}{4} \int_{l_{min}}^{l_{max}} (2l+1)dl \tag{38}$$

$$= \frac{\tilde{\chi}_a}{4} N \tag{39}$$

Substituting it in Eq. (34) yields the free energy as

$$F = \mu N + 8\pi\kappa - \frac{k_b T}{2}\left(\log N - (\bar{\sigma}+\bar{\sigma}_a)\log N + \frac{\tilde{\chi}_a}{4}N\right). \tag{40}$$

Then, the probability density in Eq. (25) becomes

$$w(N) = A\, N^{\alpha'/2} \exp[-f(N)], \tag{41}$$

where

$$f(N) = \frac{1}{k_B T}(A_1 N), \tag{42}$$

with $\alpha' = 1 - (\bar{\sigma}+\bar{\sigma}_a)$ and $A_1 = \mu - \tilde{\chi}_a/8$.

Case II: Active noise due to direct force

The direct force is exerted directly on the membrane as a random force by proteins and the cytoskeleton and described using $x_\ell = 1$ (Loubet et al., 2012). Isolating the summation term in (33) and simplifying, we get

$$\sum_{l,m} \frac{\tilde{\chi}_a x_l}{E^a_\ell} = \sum_{l,m} \kappa \frac{\tilde{\chi}_a}{q^4(\kappa - \frac{\kappa}{q^2} + \frac{\kappa(\bar{\sigma}+\bar{\sigma}_a)}{q^2})} \tag{43}$$

$$\approx \sum_{l,m} \kappa \frac{\tilde{\chi}_a}{q^4\kappa} \tag{44}$$

$$= \sum_{l,m} \frac{\tilde{\chi}_a}{l^2(l+1)^2} \tag{45}$$

$$= \int_{l_{min}}^{l_{max}} \frac{\tilde{\chi}_a}{l^2(l+1)^2}(2l+1)dl \tag{46}$$

$$= -\tilde{\chi}_a\left(\frac{1}{l_{max}^2 + l_{max}} - \frac{1}{6}\right). \tag{47}$$

Keeping the upper cut-off as $l_{max} = \frac{r_0}{d}$ (Bar-Ziv et al., 1995), and using the relation $N = \frac{4\pi r_0^2}{d^2}$ we obtain

$$\sum_{l,m} \frac{\tilde{\chi}_a x_l}{E^a_\ell} = -\tilde{\chi}_a\left(\frac{1}{\frac{N}{4\pi} + \sqrt{\frac{N}{4\pi}}} - \frac{1}{6}\right). \tag{48}$$

Then, the effective free energy for the direct force is obtained as

$$F = \mu N + 8\pi\kappa - \frac{k_b T}{2}\left(\log N - (\bar{\sigma}+\bar{\sigma}_a)\log N - \tilde{\chi}_a\left(\frac{1}{\frac{N}{4\pi} + \sqrt{\frac{N}{4\pi}}} - \frac{1}{6}\right)\right). \tag{49}$$

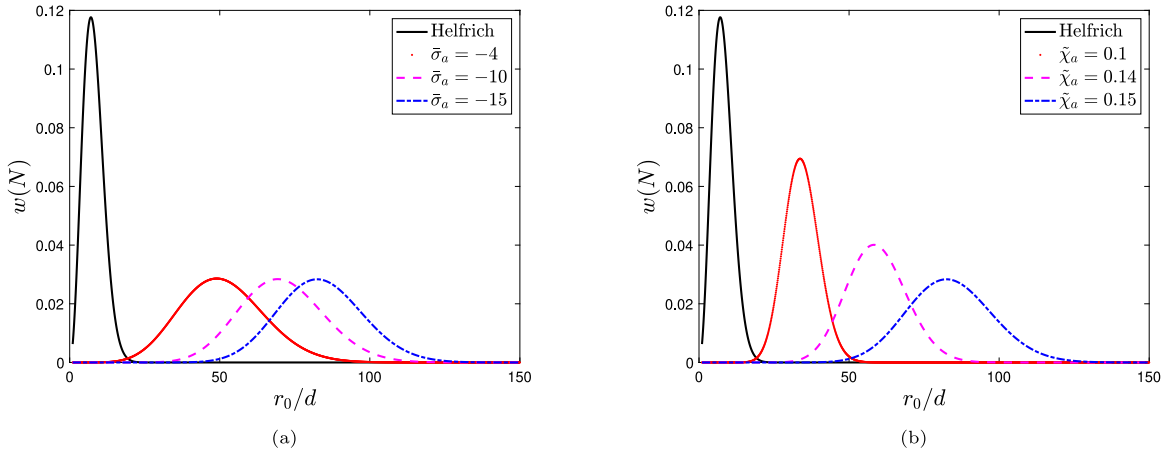

Fig. 2. Probability distribution for vesicle size as a function of r_0/d subjected to active noise due to curvature force and active tension (a) Amplitude of active noise is held constant at $\tilde{\chi}_a = 0.15$ with active tension $\bar{\sigma}_a$ varying from −15 to −4; (b) Active tension held constant at $\bar{\sigma}_a = -15$ with amplitude of active noise $\tilde{\chi}_a$ varying from 0.1 to 0.15. We use the following parameters: $\kappa = 10 k_B T$, and $d = 5 \times 10^{-9}$ m. The black curve based on the Helfrich model is drawn for reference.

Substituting Eq. (49) in Eq. (25), we get the same form for the probability distribution as Eq. (41) with

$$f(N) = \frac{1}{k_B T}\left[\mu N + A_2\left(\frac{1}{\frac{N}{4\pi} + \sqrt{\frac{N}{4\pi}}} - \frac{1}{6}\right)\right] \tag{50}$$

where $A_2 = \tilde{\chi}_a/2$ and $\alpha' = 1 - (\bar{\sigma} + \bar{\sigma}_a)$.

4. Numerical results

4.1. Interplay of active tension and noise

To elucidate how the two parameters $\bar{\sigma}_a$ and $\tilde{\chi}_a$ influence the vesicle size distribution, we present numerical results for different values of the parameters for curvature and direct force in Figs. 2 and 3. In all the figures, we plot the probability distribution obtained from the Helfrich model (Helfrich, 1986) for passive vesicles (without activity) for the sake of comparison. For simplification, we set the surface tension $\bar{\sigma}$ to zero. In our study, this implies that the membrane tension arises solely from activity and not from the energy cost for areal changes. Previous studies examining the stability of vesicles have shown that $\bar{\sigma} = -6$ is a limit for surface tension computed from the areal constraint for stability of vesicles in equilibrium (Seifert, 1999). Although active tension is not limited to such constraint, we consider values for $\bar{\sigma}_a$ on the same order. Figs. 2a and 3a reveal that active tension has significant impact on the vesicle size distribution. It makes smaller vesicle size probable by reducing the excess area available for fluctuations, and in other words, making the vesicle membrane tauter. Thus, for a constant amplitude of active noise, increase in active tension makes the size distribution shift towards smaller vesicle sizes for both curvature and direct forces. In contrast, Figs. 2b and 3b show that active noise makes larger vesicles more probable by increasing the fluctuations. Furthermore, curvature force has a more pronounced impact on vesicle size distribution than direct force. Not only does it shift the distribution to larger sizes, it also broadens the bell curve. Finally, we wish to emphasize that our model captures the threshold or cutoff for r_0/d observed in experiments even with linearized curvature elasticity.

Previously, Ahmadpoor and Sharma (2016) have reported that nonlinear curvature elasticity is important to capture this threshold for a tensionless membrane. In addition, Kulkarni (2023) has shown that only adding active noise to Helfrich's theory based on linearized curvature elasticity does not yield the threshold. This confirms that considering active tension in addition to active noise enables the model to predict the threshold and vesicle sizes that can be smaller or larger as nature deems necessary which are not possible from equilibrium considerations.

4.2. Comparison with experimental data

Finally, we compare our results with different experimental studies from literature. In our model, we have only two fitting parameters, $\bar{\sigma}_a$ and $\tilde{\chi}_a$ which are fit to experimental data concurrently using the least square fit method. Although these parameters are estimated by fitting to experimental data, they have a clear physical underpinning attributed to active physiological processes. As seen in Fig. 4, our model can fit experimental data for diverse size distributions very well. In comparison, Helfrich's model for passive vesicles is unable to capture the experimental plots. We wish to note that there is no fitting parameter in the Helfrich model presented in Fig. 4. In principle, the bending modulus can also be a fitting parameter in the Helfrich model. However, κ

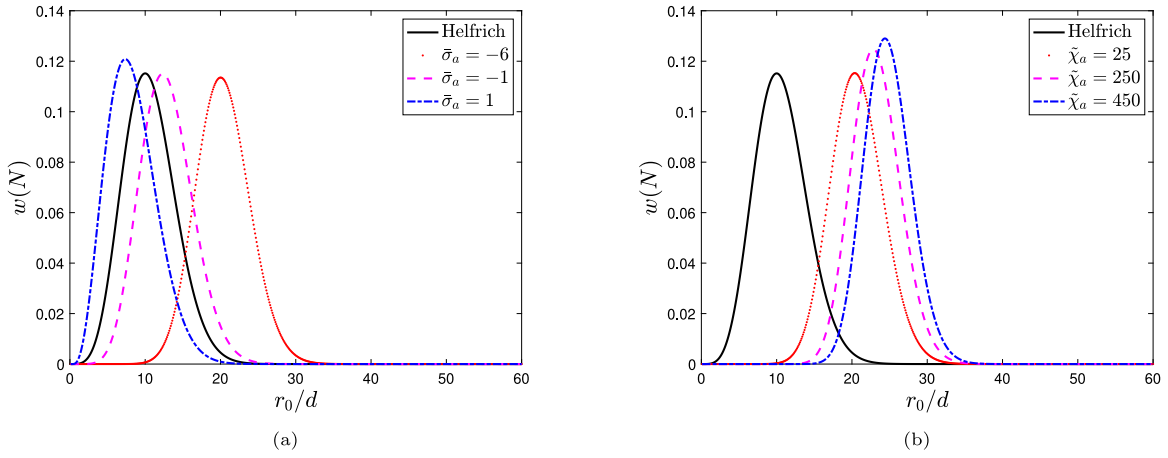

Fig. 3. Probability distribution for vesicle size as a function of r_0/d subjected to active noise due to direct force and active tension. (a) Amplitude of active noise is held constant at $\tilde{\chi}_a = 1.25$ with active tension $\bar{\sigma}_a$ varying from −6 to 1. (b) Active tension is held constant at $\bar{\sigma}_a = -6$ with amplitude of active noise $\tilde{\chi}_a$ varying from 25 to 450. We use the following parameters: $\kappa = 10k_BT$, and $d = 5 \times 10^{-9}$ m. The black curve based on the Helfrich model is drawn for reference.

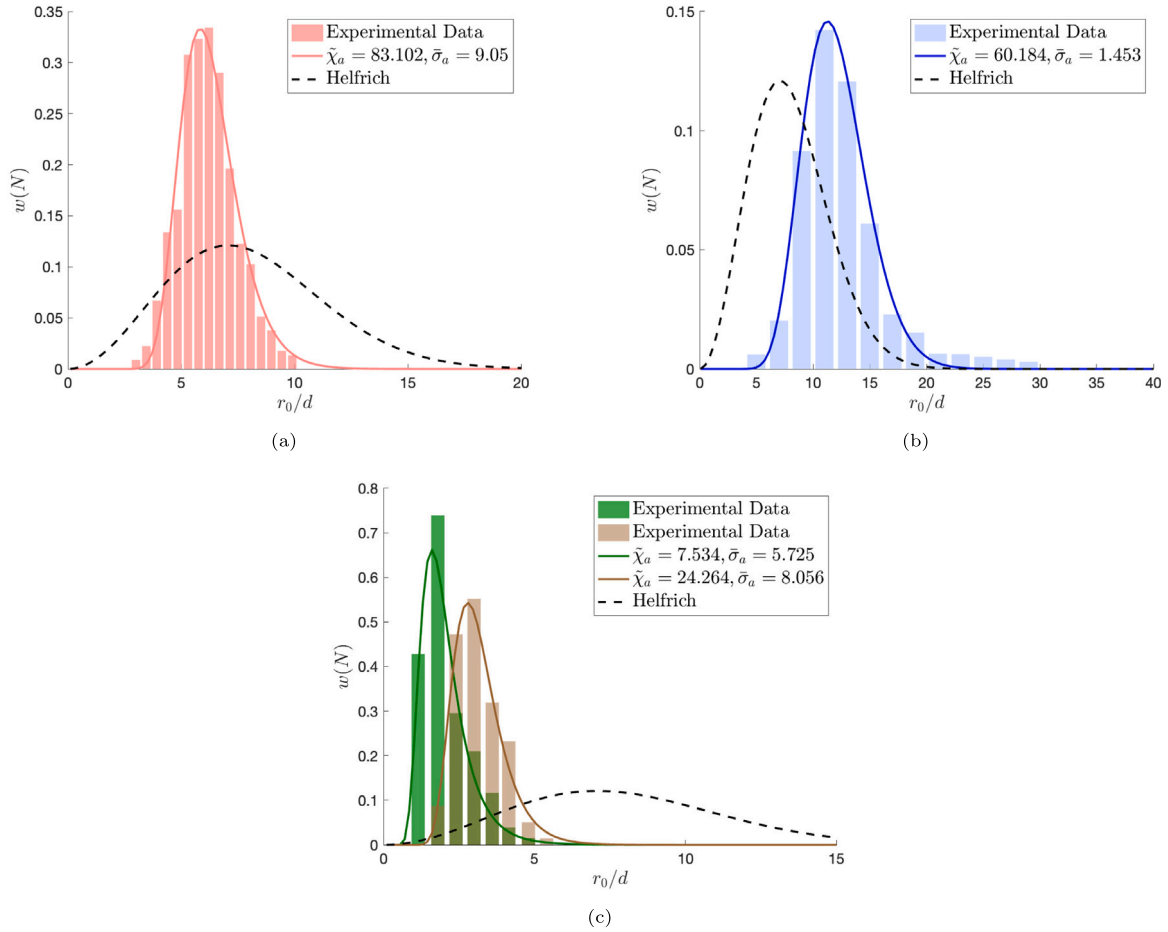

Fig. 4. Our theoretical results for size distribution (solid lines) matched with different experimental data (histograms) using least square fit. The legends show the values of $\bar{\sigma}_a$ and $\tilde{\chi}_a$ for active tension and active noise amplitude for direct force respectively. (a) shows data from Coldren et al. (2003). (b) shows data from Xu et al. (2013). (c) shows data for very small vesicle sizes from Huang et al. (2017). We used the following parameters: $\kappa = 10k_BT$, and $d = 5 \times 10^{-9}$ m for all the curves. The dashed line shows the results for the Helfrich model (Eq. (27)).

is a material property and for a lipid bilayer, it is typically in the range 10–20 K_BT. Since it does not vary significantly, simply varying κ does not offer the flexibility to fit different experimental data. This provides the motivation that the Helfrich model needs to be enriched with additional physical parameters. We emphasize that although some experimental studies have shown a wider variation of bending modulus based on effects such as salt concentration, cholesterol content and density of transmembrane proteins, the bending modulus measured in these experiments is the apparent bending modulus and corresponds to κ_{eff} rather than κ. In our model, we hypothesize that these effects are indeed accounted for by active tension and active noise and enter the size distribution through κ_{eff}. Our model proposes that active tension and active noise are both needed to capture the wide variety of experimentally observed vesicle size distributions. What our model illustrates is that vesicles made out of the same membrane (fixed bending modulus) at a given temperature can achieve different vesicle sizes by varying the activity which is not possible simply based on equilibrium considerations.

The experimental data in Fig. 4a is taken from Coldren et al. (2003). In this study, Coldren et al. report two methods to measure vesicle size distribution and speculate that the vesicle size distribution depends on a competition between the entropy of vesicles and surfactant mixing, and the curvature elasticity of the vesicle membranes. Thus, their model captures their different experimental size distributions by varying the number of amphiphilic molecules and effective bending rigidity. This is consistent with our model in which the active forces result in an effective free energy and a renormalized bending rigidity. The experimental data in Fig. 4b is taken from Xu et al. (2013). Fig. 4c shows experimentally observed size distribution for egg lecithin and egg lecithin/cholesterol vesicles taken from Huang et al. (2017). All the curves have been fitted using least square method by varying the parameters $\bar{\sigma}_a$ and $\tilde{\chi}_a$ for active tension and active noise amplitude for direct force respectively. Thus, in Fig. 4, it is the interplay of active tension and active noise that enables our model to capture the threshold or minimum vesicle diameter observed in experiments, and vary the standard deviation and mean of the Gaussian-like curve. In order to understand whether the estimates for $\bar{\sigma}_a$ and $\tilde{\chi}_a$ obtained here numerically are physically reasonable, we first note that there are no experimental measurements of active noise available in literature. There are some estimates for active noise amplitudes and surface tension provided in prior studies (Loubet et al., 2012), but they are for very large micron-sized vesicles and cannot be compared directly with values we obtain for small nanometer-sized vesicles (considered in Fig. 4) due to the orders of magnitude difference. Hence, we use energetic arguments to furnish insights into the values for active noise and tension estimated in Fig. 4 while fitting to various experimental data. To this end, we note that energy for hydrolysis of an ATP molecule, the energy currency of biological cells, is about 12–13 k_BT (Alberts et al., 2002). Using Eq. (49), we can estimate the energy contribution from the active noise term and the active tension term. For Fig. 4a which has the largest values of $\tilde{\chi}_a$ and $\bar{\sigma}_a$, for a typical vesicle with $r_0/d \approx 5$, the energy contribution for the active noise is about $5k_BT$ and that for the active tension is about $28k_BT$, which are very reasonable energetic costs for a cell to furnish using ATP hydrolysis.

5. Concluding remarks

We have investigated a model based on the principles of continuum mechanics and non-equilibrium statistical mechanics to understand the role of active membranes on the size distribution of active vesicles. Earlier studies on vesicle size distribution relied on thermal fluctuations or active fluctuations to determine size distributions. In comparison, our model is enriched with the interplay of active noise and active tension. This endows the vesicles with the ability to achieve diverse size distributions that are not captured by thermal fluctuations or active noise. Our statistical mechanics results for active vesicles with linearized curvature elasticity can reproduce different experimental data for varying vesicle size distributions published in the literature. The theoretical framework developed here opens avenues for investigating other biological problems of interest such as pore formation, and membrane inclusions where active forces may play a role. Examining the restrictions on the active tension and active noise is critical for understanding the limits on vesicle size distribution found in nature as well as synthesized in laboratories for biotechnological applications. Recently developed non-equilibrium statistical mechanics approaches (Seifert, 2012; Leadbetter et al., 2023) could provide insightful means to investigate far-from-equilibrium phenomena associated with active matter. Stability analysis of active membranes and vesicles provides another fascinating (and related) avenue for future study.

CRediT authorship contribution statement

Sreekanth Ramesh: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Yashashree Kulkarni:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

Ahmadpoor, F., Sharma, P., 2016. Thermal fluctuations of vesicles and nonlinear curvature elasticity—implications for size-dependent renormalized bending rigidity and vesicle size distribution. Soft Matter 12 (9), 2523–2536. http://dx.doi.org/10.1039/c5sm02769a.

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., Watson, J., 2002. Molecular Biology of the Cell, fourth ed. Garland.

Arroyo, M., DeSimone, A., 2009. Relaxation dynamics of fluid membranes. Phys. Rev. E 79 (3), 1–3. http://dx.doi.org/10.1103/physreve.79.031915.

Bar-Ziv, R., Frisch, T., Moses, E., 1995. Entropic expulsion in vesicles. Phys. Rev. Lett. 75 (19), 3481.

Biria, A., Maleki, M., Fried, E., 2013. Continuum theory for the edge of an open lipid bilayer. Adv. Appl. Mech. 46, 1–68. http://dx.doi.org/10.1016/b978-0-12-396522-6.00001-3.

Biswas, A., Kashyap, P., Datta, S., Sengupta, T., Sinha, B., 2019. Cholesterol depletion by MβCD enhances cell membrane tension and its variations-reducing integrity. Biophys. J. 116 (8), 1456–1468.

Bowick, M.J., Fakhri, N., Marchetti, M.C., Ramaswamy, S., 2022. Symmetry, thermodynamics, and topology in active matter. Phys. Rev. X 12, 010501. http://dx.doi.org/10.1103/PhysRevX.12.010501.

Cai, W., Lubensky, T.C., 1994. Covariant hydrodynamics of fluid membranes. Phys. Rev. Lett. 73 (8), 1186–1189. http://dx.doi.org/10.1103/physrevlett.73.1186.

Canham, P.B., 1970. The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cell. J. Theoret. Biol. 26 1, 61–81. http://dx.doi.org/10.1016/S0022-5193(70)80032-7.

Carotenuto, A.R., Lunghi, L., Piccolo, V., Babaei, M., Dayal, K., Pugno, N., Zingales, M., Deseri, L., Fraldi, M., 2020. Mechanobiology predicts raft formations triggered by ligand-receptor activity across the cell membrane. J. Mech. Phys. Solids 141, 103974. http://dx.doi.org/10.1016/j.jmps.2020.103974.

Coldren, B., van Zanten, R., Mackel, M.J., Zasadzinski, J.A., Jung, H.-T., 2003. From vesicle size distributions to bilayer elasticity via cryo-transmission and freeze-fracture electron microscopy. Langmuir 19 (14), 5632–5639.

Deseri, L., Pollaci, P., Zingales, M., Dayal, K., 2016. Fractional hereditariness of lipid membranes: Instabilities and linearized evolution. J. Mech. Behav. Biomed. Mater. 58, 11–27. http://dx.doi.org/10.1016/j.jmbbm.2015.09.021, Special issue: Mechanics of biological membranes.

Di Bella, M., 2022. Overview and update on extracellular vesicles: Considerations on exosomes and their application in modern medicine. Biology 11, 804.

Evans, E.A., 1974. Bending resistance and chemically induced moments in membrane bilayers. Biophys. J. 14 (12), 923–931. http://dx.doi.org/10.1016/S0006-3495(74)85959-X.

Farago, O., Santangelo, C.D., 2005. Pore formation in fluctuating membranes. J. Chem. Phys. 122 (4), 044901. http://dx.doi.org/10.1063/1.1835952.

Freund, L.B., 2012. Entropic pressure between biomembranes in a periodic stack due to thermal fluctuations. Proc. Natl. Acad. Sci. 110, 2047–2051. http://dx.doi.org/10.1073/pnas.1220968110.

Ginini, L., Billan, S., Fridman, E., Gil, Z., 2019. Insight into extracellular vesicle-cell communication: From cell recognition to intracellular fate. Cells 11 (9), 1375.

Girard, P., Prost, J., Bassereau, P., 2005. Passive or active fluctuations in membranes containing proteins. Phys. Rev. Lett. 94 (8), 088102.

Givli, S., Giang, H., Bhattacharya, K., 2012. Stability of MultiComponent biological membranes. SIAM J. Appl. Math. 72 (2), 489–511. http://dx.doi.org/10.1137/110831301.

Gompper, G., Kroll, D.M., 1989. Steric interactions in multimembrane systems: A Monte Carlo study. Europhys. Lett. 9 (1), 59–64. http://dx.doi.org/10.1209/0295-5075/9/1/011.

Grasinger, M., Mozaffari, K., Sharma, P., 2021. Flexoelectricity in soft elastomers and the molecular mechanisms underpinning the design and emergence of giant flexoelectricity. Proc. Natl. Acad. Sci. 118 (21), e2102477118. http://dx.doi.org/10.1073/pnas.2102477118.

Grasinger, M., Sharma, P., 2024. Thermal fluctuations (eventually) unfold nanoscale origami. J. Mech. Phys. Solids 184, 105527. http://dx.doi.org/10.1016/j.jmps.2023.105527, URL https://www.sciencedirect.com/science/article/pii/S0022509623003307.

Hanlumyuang, Y., Liu, L., Sharma, P., 2014. Revisiting the entropic force between fluctuating biological membranes. J. Mech. Phys. Solids 63, 179–186. http://dx.doi.org/10.1016/j.jmps.2013.09.011.

Helfrich, W., 1973. Elastic properties of lipid bilayers: theory and possible experiments. Z. Nat.forsch. C 26, 61–81. http://dx.doi.org/10.1515/znc-1973-11-1209.

Helfrich, W., 1978. Steric interaction of fluid membranes in multilayer systems. Z. Nat.forsch. A 33 (3), 305–315. http://dx.doi.org/10.1515/zna-1978-0308.

Helfrich, W., 1986. Size distributions of vesicles: The role of the effective rigidity of membranes. J. Physique 47 (2), 321–329. http://dx.doi.org/10.1051/jphys:01986004702032100.

Huang, C., Quinn, D., Sadovsky, Y., Suresh, S., Hsia, K.J., 2017. Formation and size distribution of self-assembled vesicles. Proc. Natl. Acad. Sci. 114 (11), 2910–2915. http://dx.doi.org/10.1073/pnas.1702065114.

Iyer, P., Gompper, G., Fedosov, D.A., 2023. Dynamic shapes of floppy vesicles enclosing active Brownian particles with membrane adhesion. Soft Matter 19, 3436–3449.

Kanno, T., Yamada, T., Iwabuki, H., Tanaka, H., Kuroda, S., Tanizawa, K., Kawai, T., 2002. Size distribution measurement of vesicles by atomic force microscopy. Anal. Biochem. 309 (2), 196–199.

Khandagale, P., Garcia-Cervera, C., deBotton, G., Breitzman, T., Majidi, C., Dayal, K., 2024. Statistical field theory of polarizable polymer chains with nonlocal dipolar interactions. Phys. Rev. E 109, 044501. http://dx.doi.org/10.1103/PhysRevE.109.044501, URL https://link.aps.org/doi/10.1103/PhysRevE.109.044501.

Kleinert, H., 1986. Size distribution of spherical vesicles. Phys. Lett. A 116 (2), 57–62. http://dx.doi.org/10.1016/0375-9601(86)90238-0.

Korgel, B.A., Van Zanten, J.H., Monbouquette, H.G., 1998. Vesicle size distributions measured by flow field-flow fractionation coupled with multiangle light scattering. Biophys. J. 74 (6), 3264–3272.

Kulkarni, Y., 2023. Fluctuations of active membranes with nonlinear curvature elasticity. J. Mech. Phys. Solids 173, 105240.

Kumar, M., et al., 2024. Extracellular vesicles as tools and targets in therapy for diseases. In: Signal Transduction and Targeted Therapy. Vol. 9, pp. 2059–3635.

Leadbetter, T., Purohit, P.K., Reina, C., 2023. A statistical mechanics framework for constructing nonequilibrium thermodynamic models. PNAS Nexus 2 (12), pgad417. http://dx.doi.org/10.1093/pnasnexus/pgad417.

Lee, A.A., Vella, D., Wettlaufer, J.S., 2017. Fluctuation spectra and force generation in nonequilibrium systems. Proc. Natl. Acad. Sci. 114 (35), 9255–9260. http://dx.doi.org/10.1073/pnas.1701739114.

Liao, X., Purohit, P.K., 2021. Kinetics of self-assembly of inclusions due to lipid membrane thickness interactions. Soft Matter 17 (9), 2539–2556. http://dx.doi.org/10.1039/D0SM01752C.

Liao, X., Purohit, P.K., Gopinath, A., 2020. Extensions of the worm-like-chain model to tethered active filaments under tension. J. Chem. Phys. 153 (19), 194901. http://dx.doi.org/10.1063/5.0025200.
Lipowsky, R., 1991. The conformation of membranes. Nature 349, 475. http://dx.doi.org/10.1038/349475a0.
Liu, L.P., Sharma, P., 2013. Flexoelectricity and thermal fluctuations of lipid bilayer membranes: Renormalization of flexoelectric, dielectric, and elastic properties. Phys. Rev. E 87, 032715. http://dx.doi.org/10.1103/PhysRevE.87.032715.
Lomholt, M.A., 2006. Fluctuation spectrum of quasispherical membranes with force-dipole activity. Phys. Rev. E 73 (6), 061914. http://dx.doi.org/10.1103/physreve.73.061914.
Loubet, B., Seifert, U., Lomholt, M.A., 2012. Effective tension and fluctuations in active membranes. Phys. Rev. E 85 (3), 031913. http://dx.doi.org/10.1103/physreve.85.031913.
Maitra, A., Srivastava, P., Rao, M., Ramaswamy, S., 2014. Activating membranes. Phys. Rev. Lett. 112 (25), 258101. http://dx.doi.org/10.1103/physrevlett.112.258101.
Men, Y., Peng, F., Tu, Y., van Hest, J.C.M., Wilson, D.A., 2016. Methods for production of uniform small-sized polymersome with rigid membrane. Polym. Chem. 7, 3977–3982.
Menger, F.M., Lee, J.-J., Aikens, P., Davis, S., 1989. Vesicle size: dependence on experimental parameters. J. Colloid Interface Sci. 129 (1), 185–191.
Milner, S.T., Safran, S.A., 1987. Dynamical fluctuations of droplet microemulsions and vesicles. Phys. Rev. A 36 (9), 4371–4379. http://dx.doi.org/10.1103/physreva.36.4371.
Nelson, D.R., Piran, T., Weinberg, S., 2004. Statistical Mechanics of Membranes and Surfaces. World Scientific Publishing Co., Singapore.
Nguyen, T.D., Mao, S., Yeh, Y.-W., Purohit, P.K., McAlpine, M.C., 2013. Nanoscale flexoelectricity. Adv. Mater. 25 (7), 946–974. http://dx.doi.org/10.1002/adma.201203852.
Phillips, R., Kondev, J., Theriot, J., Garcia, H., 1998. Physical Biology of the Cell. Garland Science, Boca Raton, http://dx.doi.org/10.1201/9781134111589.
Prost, J., Bruinsma, R., 1996. Shape fluctuations of active membranes. Europhys. Lett. 33 (4), 321–326. http://dx.doi.org/10.1209/epl/i1996-00340-1.
R Mozafari, M., Linder, M., 2011. Liposomes: a review of manufacturing techniques and targeting strategies. Curr. Nanosci. 7 (3), 436–452.
Ramaswamy, S., 2010. The mechanics and statistics of active matter. Annu. Rev. Condens. Matter Phys. 1 (1), 323–345. http://dx.doi.org/10.1146/annurev-conmatphys-070909-104101.
Ramaswamy, S., Rao, M., 2001. The physics of active membranes. C. R. l'Acad. Sci. Ser. IV 2 (6), 817–839. http://dx.doi.org/10.1016/s1296-2147(01)01226-4.
Ramaswamy, S., Toner, J., Prost, J., 2000. Nonequilibrium fluctuations, traveling waves, and instabilities in active membranes. Phys. Rev. Lett. 84 (15), 3494–3497. http://dx.doi.org/10.1103/physrevlett.84.3494.
Roffay, C., Molinard, G., Kim, K., Urbanska, M., Andrade, V., Barbarasa, V., Nowak, P., Mercier, V., García-Calvo, J., Matile, S., et al., 2021. Passive coupling of membrane tension and cell volume during active response of cells to osmosis. Proc. Natl. Acad. Sci. 118 (47), e2103228118.
Ronquist, G.K., 2019. Extracellular vesicles and energy metabolism. Clin. Chim. Acta 488, 116–121.
Safran, S., 1994. Statistical Thermodynamics of Surfaces, Interfaces, and Membranes. Perseus Books, New York.
Santangelo, C.D., Farago, O., 2007. Membrane fluctuations around inclusions. J. Comput. Aided Mater. Des. 14 (1), 103–109. http://dx.doi.org/10.1007/s10820-007-9081-x.
Saric, A., Freeman, S.A., 2021. Solutes as controllers of endomembrane dynamics. Nat. Rev. Mol. Cell Biol. 22 (4), 237–238.
Schneider, M., Jenkins, J., Webb, W., 1984. Thermal fluctuations of large quasi-spherical bimolecular phospholipid vesicles. J. Physique 45 (9), 1457–1472. http://dx.doi.org/10.1051/jphys:019840045090145700.
Seifert, U., 1999. Fluid membranes in hydrodynamic flow fields: Formalism and an application to fluctuating quasispherical vesicles in shear flow. Eur. Phys. J. B 8 (3), 405–415. http://dx.doi.org/10.1007/s100510050706.
Seifert, U., 2012. Stochastic thermodynamics, fluctuation theorems and molecular machines. Rep. Progr. Phys. 75 (12), 126001. http://dx.doi.org/10.1088/0034-4885/75/12/126001.
Steigmann, D.J., 2018. The Role of Mechanics in the Study of Lipid Bilayers. Springer International Publishing, Cham.
Turlier, H., Betz, T., 2018. Fluctuations in active membranes. In: Bassereau, P., Sens, P. (Eds.), Physics of Biological Membranes. Springer International Publishing, Cham, pp. 581–619. http://dx.doi.org/10.1007/978-3-030-00630-3_21.
Turlier, H., Betz, T., 2019. Unveiling the active nature of living-membrane fluctuations and mechanics. Annu. Rev. Condens. Matter Phys. 10 (1), 213–232. http://dx.doi.org/10.1146/annurev-conmatphys-031218-013757.
Xu, W., Wang, X., Zhong, Z., Song, A., Hao, J., 2013. Influence of counterions on lauric acid vesicles and theoretical consideration of vesicle stability. J. Phys. Chem. B 117 (1), 242–251.
Zelisko, M., Ahmadpoor, F., Gao, H., Sharma, P., 2017. Determining the Gaussian modulus and edge properties of 2D materials: From graphene to lipid bilayers. Phys. Rev. Lett. 119, 068002. http://dx.doi.org/10.1103/PhysRevLett.119.068002.
Zhang, G., Sun, J., 2021. Lipid in chips: A brief review of liposomes formation by microfluidics. Int. J. Nanomed. 16 (3), 7391–7416.
Zhong-can, O.-Y., Helfrich, W., 1989. Bending energy of vesicle membranes: General expressions for the first, second, and third variation of the shape energy and applications to spheres and cylinders. Phys. Rev. A 39 (10), 5280–5288. http://dx.doi.org/10.1103/physreva.39.5280.