



Fluctuations of active membranes with nonlinear curvature elasticity

Yashashree Kulkarni

Department of Mechanical Engineering, University of Houston, Houston, TX 77204, USA

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ABSTRACT

Biological membranes and vesicles play a vital role in critical physiological processes like endocytosis, cell division, and cell motility. Over decades, statistical and continuum mechanics studies have provided phenomenal insights into the mechanics of these membranes and their biophysical implications. However, most studies, until recently, have focused entirely on passive (or “dead”) membranes that exhibit only equilibrium thermal fluctuations. In this work, we acknowledge the growing consensus that the active nature of membranes is vital to their biophysical functioning. Active membranes contain proteins that are fueled by energy from external sources such as adenosine triphosphate hydrolysis or light. These proteins exert forces on the membrane during their activity causing the membrane to exhibit fluctuations that are non-thermal in origin, thus driving them away from equilibrium. In short, active membranes are “alive” with their own energy source capable of circumventing equilibrium considerations. In this paper, we present a theory for active membranes based on principles of continuum mechanics and a variational formulation that (we hope) provides a unifying framework to understand seemingly disparate but insightful prior works in this field. Using our developed continuum model, we study the statistical mechanics of active closed membranes or vesicles. A specific highlight of our work is that we incorporate nonlinear curvature elasticity in our continuum theory and obtain closed-form results for the fluctuation spectra for active vesicles. Our numerical results reveal a rather unanticipated interplay of active forces, surface tension as well as nonlinear elasticity for small vesicles. To gain insights into the biophysical implications of activity in vesicles, we revisit the problem of determining the vesicle size distribution by assuming that the active vesicles are close to thermal equilibrium. Our numerical calculations show that active forces may significantly impact vesicle size distribution. A tantalizing implication of this finding is that by tuning active forces, active vesicles may attain vesicle size distributions that are improbable for passive vesicles governed only by thermal fluctuations.

1. Introduction

Biological membranes form the interface that separates cells (or their internal organelles and vesicles) from their environment. As these cellular entities constantly interact with the surrounding medium, biological membranes play a central role in all cellular functions and physiological processes. Whether it is the response of cells to mechanical stimuli, transmission of messages through electrochemical signals, exchange of nutrients or expelling of waste product, biological membranes are inevitably involved in these crucial processes.

The field of continuum mechanics and equilibrium statistical mechanics has made remarkable inroads into the understanding of biological media and the literature, spanning several decades, is fantastically rich (see [Lipowsky, 1991](#); [Safran, 1994](#); [Nelson et al.,](#)

E-mail address: ykulkarni@uh.edu.

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2004; Steigmann, 2018; Seifert, 1997; Ahmadpoor and Sharma, 2017 for a bird's-eye view of the field). In particular, studies on equilibrium thermal fluctuations of biological membranes have led to some extraordinary insights into the role of mechanics in a host of biophysics problems such as entropic interactions between fluctuating membranes (Helfrich, 1978; Gompper and Kroll, 1989; Freund, 2012; Hanlmyuang et al., 2014), effect of edges (Biria et al., 2013; Zelisko et al., 2017), thermal fluctuations of vesicles (Helfrich, 1973, 1986; Milner and Safran, 1987; Kleinert, 1986; Seifert, 1995), pore formation in membranes (Farago and Santangelo, 2005), pinned membranes (Gov and Safran, 2004; Janeš et al., 2019), membrane inclusions and interactions (Santangelo and Farago, 2007; Agrawal et al., 2016; Liang and Purohit, 2018; Carotenuto et al., 2020; Liao and Purohit, 2021) stability of membranes (Zhong-can and Helfrich, 1989; Givli et al., 2012; Deseri et al., 2016) and electromechanical coupling (Liu and Sharma, 2013; Nguyen et al., 2013; Torbati et al., 2022) among many others. Some recent studies have also modeled biological membranes using various computational approaches (Yuan et al., 2010; Rim et al., 2014; Liang and Purohit, 2016; Madenci et al., 2020). A common theme in all the above studies is the reliance on linearized membrane elasticity based on the classical bending energy for a fluid membrane proposed by Helfrich (1973). A continuum mechanics based approach has also enabled a systematic consideration of nonlinear elasticity in the study of membrane mechanics (Steigmann, 2009; Ahmadpoor et al., 2017). Ahmadpoor and Sharma (2016) have shown that nonlinear curvature elasticity enables an explanation of a size-dependent renormalization of the bending rigidity especially for small vesicle sizes. Their results also reveal a cut-off radius for the vesicle size distribution consistent with experiments but not captured by linear elasticity models (Helfrich, 1986). Most of these studies, until recently, have focused entirely on the treatment of biological membranes as *passive* membranes. Here, the term *passive* refers to membranes that are in thermal equilibrium. Thus, they exhibit only fluctuations that arise from thermal vibrations of membrane molecules, or in some sense, are “dead”.

In contrast, *active* processes are an essential part of what defines living matter. Here, the term *active* refers to the quality of biological matter to harness energy from an intrinsic or extrinsic energy source to execute specific biological functions (Ramaswamy and Rao, 2001; Ramaswamy, 2010; Bowick et al., 2022). Prominent examples of active matter at the cellular scale are the so-called active membranes. These active membranes contain active proteins that aid in the trafficking of molecules such as ions, lipids or proteins, across cellular membranes as part of a variety of vital physiological processes such as endocytosis, cell division, cell motility, or homeostasis. The proteins are often recruited at precise locations and times on cellular membranes to perform specific operations and are fueled by energy derived from an external source. 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 (ATP) hydrolysis, mechanical stresses, electric fields or directly from light. These forces in turn cause the membranes to exhibit *active* fluctuations that are distinct from *passive* or equilibrium thermal fluctuations.

This leads to an intriguing question: how do active fluctuations impact membrane morphology, and dynamics? We seek answer to this question by looking at the fascinating case of flickering of red blood cells first observed more than a century ago, in 1890 (Browicz, 1890; Turlier and Betz, 2018). The flickering was initially thought to be a result of thermal fluctuations, akin to Brownian motion of microscopic particles. Only recently, substantial experimental evidence has pointed to an active origin of the flickering of red blood cells. In one study (Blowers et al., 1951), the flickering was observed to be correlated to ion transport driven by ATP hydrolysis which implied that the flickering was a manifestation of active fluctuations. A recent experimental study (Turlier et al., 2016) provided compelling evidence for the active nature of red blood cell flickering by showing that the flickering directly violates equilibrium statistical mechanics, proving the presence of non-equilibrium active forces driving membrane movement. Thus, the capability to measure active fluctuations and distinguish them from passive thermal fluctuations using modern experimental techniques conclusively resolved this 125 year old controversy.

It is now well-established that there can be a variety of sources for active forces. In the last two decades, active membrane fluctuations originating from ion pumps have been the focus of pioneering biophysics studies, both from theoretical, and experimental perspectives (Prost and Bruinsma, 1996; Manneville et al., 1999; Ramaswamy et al., 2000; Lin et al., 2006; Loubet et al., 2012; Lacoste and Bassereau, 2014; Turlier and Betz, 2019). These studies show that the activity of ion pumps leads to significant modifications in the fluctuation properties of vesicles, and can be measured as changes in the fluctuation amplitude, in the effective membrane tension or in the excess surface area. Fig. 1 shows a simple illustration of the observable difference between passive and active membranes. When both are subjected to an external energy source, the active proteins embedded in active membranes are switched “on” and enhance the fluctuations of the active membrane due to their activity whereas the passive membrane continues to exhibit thermal fluctuations. In fact, experimental measurements have revealed that the variation of the surface tension with change in excess area is noticeably different for passive and active membranes indicating the contribution from active fluctuations in the latter. Besides ATP or photon driven ion pumps, lipid transport systems such as flippases may also contribute to active fluctuations (Hankins et al., 2015), as well as membrane-fusion and fission of transport vesicles (Rao and Sarasij, 2001). Active fluctuations can also originate from the interaction of the membrane with the underlying cytoskeleton, such as the spectrin network or the actomyosin cortex (Fournier et al., 2004; Fodor et al., 2015). The cytoskeleton may exert tangential and normal forces on the membrane under the action of molecular motors, or via polymerization of filaments. A recent experimental and numerical study has shown that non-equilibrium fluctuations can also be caused by bacteria-membrane contact forces (Takatori and Sahu, 2020).

Recently, Liao et al. (2020) have studied the fluctuations of “active” filaments. It is interesting to note that their results for active fluctuations of a one-dimensional chain model are analogous to those obtained for two-dimensional membranes. Buskermolen et al. (2019) identified active fluctuations and concomitant movement of cells under the action of active forces and proposed a novel approach based on maximizing the entropy subject to certain constraints arising from activity of the cell. In this regard, Huang et al. (2021) propose an interesting theoretical and computational approach for using fluctuation theorems to understand non-equilibrium phenomena which might provide a possible avenue for modeling active matter.

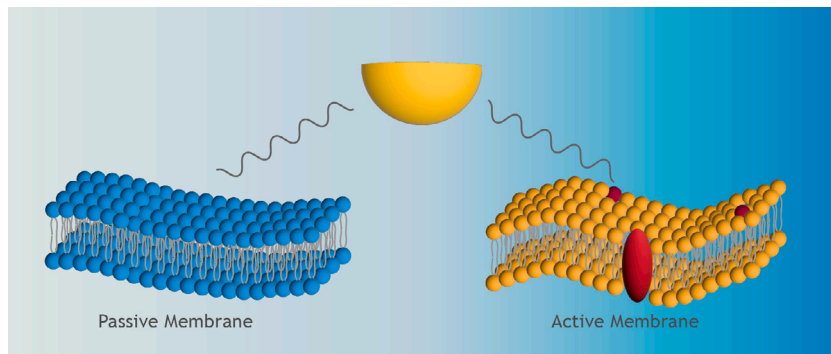


Fig. 1. Schematic to illustrate the difference between passive and active membranes. When subjected to an external energy source, active proteins exert non-thermal forces that dramatically enhance the fluctuations of active membranes compared to passive membranes which exhibit thermal fluctuations only.

All these studies, and many others, point to the growing consensus in the scientific community that it is imperative to develop an understanding of active matter for a more complete understanding of biological phenomena and processes, which entails going beyond equilibrium statistical mechanics. They also highlight the vital role of mechanics in quantifying the signature of activity in cells, vesicles, and membranes and associated cellular functions.

Many different statistical mechanics approaches have been developed to study systems that are not in thermal equilibrium and the literature spans several decades (Prigogine, 2017; Zubarev, 1974; Dougherty, 1994; Pathria and Beale, 2011; Sethna, 2006). Our approach is rooted in the Langevin theory of Brownian motion which involves solving equations of motion that describe the evolution of a system driven by random forces. This approach falls under a broader category of stochastic methods that focus on the role of fluctuations in non-equilibrium systems that are near equilibrium. The framework is predicated on the fluctuation–dissipation theorem to study how energy is dissipated and how work is done in systems that are driven away from equilibrium. Approaches based on the master equation form another class of non-equilibrium statistical mechanics methods which involve a set of differential equations that describe the time evolution of the probability distribution of the system. Fokker–Planck equation is a special case of the master equation which assumes a continuous probability distribution for a dissipative system and hence yields a partial differential equation which is often easy to work with in cases where directly solving equations of motion is not feasible. Although these methods have proved to be useful in studying systems close to equilibrium, they have demonstrated success in modeling systems far from equilibrium on a case by case basis. A significantly different approach is the so-called maximum caliber approach which presents a variational principle for non-equilibrium processes and focuses on probabilities of pathways or trajectories instead of states (Jaynes, 1980; Ghosh et al., 2020). Finally, computational methods, such as molecular dynamics or Monte Carlo methods, have gained rapid prominence in recent years in understanding non-equilibrium behavior especially for their value in going far from equilibrium, although computational constraints have hindered their success in simulating non-equilibrium problems.

The goal of this paper is to develop an understanding of the mechanics of active membranes from a continuum mechanics viewpoint. To this end, we address the following subjects in this paper:

- We present a continuum theory to develop the non-equilibrium statistical mechanics of active membranes based on a variational formulation. Although studies considering the dynamics of active fluid membranes may be found in the physics literature starting with the pioneering work of Prost and Bruinsma (1996), our approach is strongly connected to principles of continuum mechanics. The general theory developed here provides a unifying “mechanics-based” framework to understand and build upon seemingly disparate but insightful prior works in this field. The framework will also facilitate extending the theory to biophysics problems not considered in this work such as entropic interactions between active membranes, inclusion–inclusion interactions in active membranes, or interplay of electromechanical coupling and activity in membranes.
- A specific highlight of our study is that we incorporate nonlinear curvature elasticity in the continuum theory for active membranes. Existing treatment of active fluctuations describes membranes using the classical quadratic Helfrich–Canham hamiltonian. Recently, Ahmadpoor and Sharma (2016) have showed that nonlinear considerations gain prominence especially in the context of large-curvature or small-sized (passive) vesicles in the absence of surface tension. Here, we investigate the role of nonlinear curvature elasticity in vesicles made of active membranes. We obtain a surprising, albeit convenient, result that despite the nonlinear nature of the problem, the over-damped Langevin equation for a quasi-spherical vesicle remains linear and hence amenable to solutions found in the literature. The nonlinear curvature elasticity simply modifies the coefficients in the Langevin equation. Our model also reveals that nonlinear curvature elasticity renormalizes the surface tension.
- Using our continuum theory for active membranes, we study the statistical mechanics of active quasi-spherical vesicles with nonlinear curvature elasticity. We derive closed-form results for their fluctuation spectra. We present numerical results for a certain class of “curvature-inducing” and “direct” active forces to elucidate the interplay of activity, surface tension, and nonlinear curvature elasticity, especially in the case of smaller vesicles. To understand the implication of activity, we reexamine the problem of vesicle size distribution assuming active vesicles to be in thermal equilibrium. These preliminary numerical results illustrate that active vesicles may attain vesicle size distributions that are deemed improbable for passive vesicles governed only by thermal fluctuations.

The outline of the paper is as follows. We begin with a brief overview of the well-established field of equilibrium statistical mechanics of membranes in Section 2. The intent of this section is to distinguish between the equilibrium statistical mechanics treatment of passive membranes and non-equilibrium statistical mechanics considerations required for active membranes. In Section 3, we establish the general theoretical framework for active membranes based on principles of continuum mechanics and variational formulation. As an illustrative example, we apply the theory to quasi-planar membranes and derive closed-form expressions for fluctuation spectra for passive and active membranes. Section 4 presents the statistical mechanics of passive and active closed membranes (vesicles) based on our continuum model. We derive closed-form results for active vesicles with nonlinear curvature elasticity. We present numerical results for fluctuation spectra for vesicles subjected to different types of active forces and discuss the interplay between activity, surface tension and nonlinear elasticity. In Section 5, we revisit the problem of vesicle size distribution to furnish insights into the effect of active fluctuations in comparison with thermal fluctuations. We close our paper with Section 6 which summarizes the highlights of the work and discusses avenues for future studies.

2. A brief review of continuum mechanics of passive membranes

2.1. Mathematical preliminaries and general framework

In continuum mechanics, membranes are described as elastic sheets that are resistant to change in area or in-plane deformations, but can bend easily with out-of-plane deformation (Phillips et al., 1998). Mathematically, we represent the membrane as a smooth orientable surface \mathbb{S} in three-dimensional space (Fig. 2). Let \mathbf{n} be a unit normal field on \mathbb{S} . The surface projection tensor can be defined as

$$\mathbf{P} = \mathbf{I} - \mathbf{n} \otimes \mathbf{n}, \tag{1}$$

with \mathbf{I} being the three-dimensional identity tensor. Given scalar- and vector-valued fields f and \mathbf{g} , their surface gradients $\nabla_S f$ and $\nabla_S \mathbf{g}$ are defined using three-dimensional gradients ∇f^e and $\nabla \mathbf{g}^e$

$$\nabla_S f = \mathbf{P} \nabla f^e, \quad \nabla_S \mathbf{g} = \mathbf{P} \nabla \mathbf{g}^e, \tag{2}$$

where f^e and \mathbf{g}^e are smooth extensions of superficial fields f and \mathbf{g} . Similarly, the surface divergence div_S and surface Laplacian Δ_S are defined as

$$\text{div}_S \mathbf{g} = \mathbf{P} \cdot \nabla \mathbf{g}^e, \quad \Delta_S f = \text{div}_S(\nabla_S f). \tag{3}$$

Then, the curvature tensor can be expressed as (Biria et al., 2013)

$$\mathbf{L} = -\text{div}_S \mathbf{n}, \tag{4}$$

and the mean curvature H , and the Gaussian curvature K are defined as

$$H = \frac{1}{2} \text{tr} \mathbf{L}, \quad K = \frac{1}{2} [(\text{tr} \mathbf{L})^2 - \text{tr} \mathbf{L}^2]. \tag{5}$$

Specifically, biological membranes are regarded as fluid membranes that typically possess only bending elastic energy. In-plane stretching is uncoupled with bending deformation. Thus, the total potential energy of the membrane is defined as (Biria et al., 2013)

$$\mathcal{F} = \int_{\mathbb{S}} \bar{\psi}(H, K) d\mathbb{S}, \tag{6}$$

where $\psi = \bar{\psi}(H, K)$ is the elastic energy density and is a function of the mean curvature H , and the Gaussian curvature K . In order to accommodate the constraint on area, we consider the augmented functional

$$\mathcal{F} = \int_{\mathbb{S}} (\psi + \sigma) d\mathbb{S}, \tag{7}$$

where σ is the Lagrange multiplier associated with the areal constraint. Physically, σ is the surface tension in the membrane that describes the energy cost for change in membrane area due to deformation. Furthermore, most studies based on linearized curvature elasticity assume the elastic energy density to be quadratic and expressed using the renowned Helfrich–Canham–Evans Hamiltonian (Canham, 1970; Helfrich, 1973; Evans, 1974),

$$\bar{\psi}(H, K) = \frac{1}{2} \kappa (H - H_0)^2 + \bar{\kappa} (K - K_0), \tag{8}$$

where κ and $\bar{\kappa}$ are the bending moduli that represent the energetic costs associated with changes in H and K respectively. H_0 and K_0 denote the corresponding spontaneous curvatures.

If we consider an infinite quasi-planar membrane, that is, an infinite membrane fluctuating around a mean flat position, a convenient description is based on the Monge parametrization. Here, the out-of-plane deformation is expressed in terms of the local height $h(\mathbf{r})$ where $\mathbf{r} = (x, y)^T$ is the position vector in the flat reference configuration. That is, the three-dimensional position vector of a point on the membrane is given by $\mathbf{R} = (x, y, h(\mathbf{r}))^T$. The unit normal vector and the areal Jacobian can be written as,

$$\mathbf{n} = \frac{1}{\sqrt{1 + |\nabla h(\mathbf{r})|^2}} (-\nabla h(\mathbf{r}), 1), \quad J = \sqrt{1 + |\nabla h(\mathbf{r})|^2}. \tag{9}$$

Consequently, the mean and Gaussian curvatures take the form (Deserno, 2015; Torbati et al., 2022)

$$H = \nabla \cdot \left(\frac{\nabla h(\mathbf{r})}{\sqrt{1 + |\nabla h(\mathbf{r})|^2}} \right), \quad K = \frac{\det(\nabla \nabla h(\mathbf{r}))}{(1 + |\nabla h(\mathbf{r})|^2)^2}. \tag{10}$$

Under small gradient approximation, $\nabla h \ll 1$,

$$H \approx \Delta h(\mathbf{r}). \tag{11}$$

According to the Gauss–Bonnet theorem (Kreuzsig, 1991), for a surface with no boundaries and in the absence of topological changes, the contribution to the membrane potential energy from the Gaussian curvature vanishes. Then, neglecting spontaneous curvature and the contribution from Gaussian curvature in Eq. (8), the total potential energy given by Eq. (7) becomes,

$$F = \frac{1}{2} \int_{S_0} dx dy [\kappa(\Delta h)^2 + \sigma(\nabla h)^2] \tag{12}$$

, where S_0 is the flat parametric domain. Under static equilibrium, an elastic sheet will occupy a configuration that minimizes this potential energy. In fact, under zero spontaneous curvature and no external forces, it will occupy a flat, undeformed configuration. However, this description holds true only at zero Kelvin temperature.

2.2. Fluctuations of passive membranes — equilibrium analysis

At finite temperature, there is a finite probability of finding the membrane away from the perfectly flat configuration given by the Boltzmann formula (Sethna, 2006),

$$p_i \propto \exp(-E_i/k_B T). \tag{13}$$

Here, p_i is the probability of the i th state, E_i is the associated energy, k_B is the Boltzmann constant, and T is the temperature. Hence, a membrane always exhibits thermal fluctuations around its static equilibrium configuration at non-zero temperatures. The probability distribution is typically normalized by defining the partition function Z as the sum of probabilities of all modes,

$$Z = \sum_i \exp(-E_i/k_B T). \tag{14}$$

Using a Fourier series representation for the height function, $h(\mathbf{r}) = \sum_{\mathbf{q}} h_{\mathbf{q}} e^{i\mathbf{q}\cdot\mathbf{r}}$ in Eq. (12), we get the potential energy in Fourier space as (Deserno, 2015)

$$F = \frac{A}{2} \sum_{\mathbf{q}} |h_{\mathbf{q}}|^2 [\kappa q^4 + \sigma q^2], \tag{15}$$

where A is the area of the flat membrane. $q = |\mathbf{q}|$ is the magnitude of the wave vector \mathbf{q} introduced in the Fourier series representation. The statistical (ensemble) average of any physical quantity Φ , with value Φ_i in the i th state, is determined using the Boltzmann distribution

$$\langle \Phi \rangle = Z^{-1} \sum_i \Phi_i \exp(-E_i/k_B T). \tag{16}$$

Since the energy specified in Eq. (12) is quadratic, the ensemble average can be computed analytically. Alternatively, we take recourse in a fundamental result of equilibrium statistical mechanics, the equipartition theorem, which states that each mode contributes $k_B T/2$ to the total energy when the energy is quadratic and hence the modes are harmonic and uncoupled (Sethna, 2006). This directly gives us the fluctuation spectrum for the membrane as (Helfrich, 1975)

$$\langle |h_{\mathbf{q}}|^2 \rangle = \frac{k_B T}{A(\kappa q^4 + \sigma q^2)}. \tag{17}$$

This formulation provides an elegant means to determine membrane properties such as bending rigidity from fluctuations data obtained from experiments, and possibly molecular simulations. Since surface tension σ is introduced as a Lagrange multiplier for the area constraint, it is determined by measuring the change in area. We also wish to note that it is straightforward to apply the above derivation based on equilibrium statistical mechanics to vesicles and the fluctuation spectra is obtained in terms of spherical harmonics (Helfrich, 1986).

Thus, combining the tools of continuum mechanics and equilibrium statistical mechanics furnishes a powerful way to extract the mechanical properties of membranes simply by analyzing their thermal fluctuations. These methods have been successfully applied in biology (Safran, 1994; Nelson et al., 2004) as well as materials science (Ahmadpoor and Sharma, 2017; Hoyt et al., 2010; Chen and Kulkarni, 2017, 2013; Ahmadpoor et al., 2017).

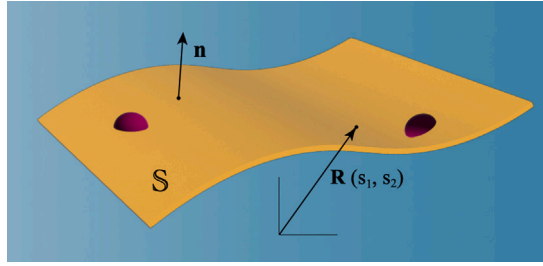


Fig. 2. Schematic of a smooth orientable surface \mathbb{S} in three-dimensional space. $\mathbf{R}(s_1, s_2)$ denotes the position vector of a point on the surface parametrized by coordinates (s_1, s_2) fluctuating in a three-dimensional fluid. \mathbf{n} denotes the normal to the surface at a given point.

3. A continuum theory for active membranes

In this section, we present a continuum theory for active membranes. The key distinction from the preceding section is that due to the non-thermal origins of active fluctuations, we need to employ tools from non-equilibrium statistical mechanics and consider the dynamics of the membrane fluctuating in a viscous medium. Although studies considering the dynamics of fluid membranes are found in biophysics literature, and we recover their results for the statistical mechanics of active membranes, our approach is strongly connected to principles of continuum mechanics and presents a unifying framework for the study of active membranes.

In Section 3.1, we present the general framework for the continuum theory based on a variational formulation. Our interest is primarily in the evolution of the membrane in the viscous medium. However, this problem is not amenable to an analytical solution. Following the numerous prior studies on dynamics of fluid membranes (Turlier and Betz, 2018), the issue is circumvented by solving the Stokes’s equation for the embedding fluid and recovering the equation for the dynamics of the membrane by applying appropriate traction and displacement boundary conditions on the fluid-membrane interface.

In Sections 3.2 and 3.3, we use quasi-planar membranes as illustrative examples and respectively summarize the statistical mechanics of passive and active membranes derived from the continuum model.

3.1. General framework

Consider a two-dimensional fluid membrane occupying a domain \mathbb{S} and embedded in a Newtonian incompressible fluid with viscosity η . For convenience, the fluid is assumed to occupy three-dimensional infinite space. Let $\mathbf{R}(s_1, s_2)$ be the position vector of a point on the membrane parametrized by (s_1, s_2) and \mathbf{x} be the position vector of a point in the three-dimensional bulk fluid.

Consistent with prior studies, we only consider viscous dissipation in the embedding fluid as it is generally understood to be the dominant dissipation mechanism (Seifert, 1999; Turlier and Betz, 2018; Lacoste and Bassereau, 2014). The membrane is assumed to be impermeable to the fluid.

Because the system of interest, the cellular fluid, belongs to the low Reynolds number regime, inertial forces are negligible. Then, the governing equations for the fluid can be derived based on Onsager’s variational principle which yields the familiar Stokes equations for hydrodynamics. Denoting the velocity of a fluid particle with position vector $\mathbf{x} \in \mathbb{R}^3$ by $\mathbf{V}(\mathbf{x})$, the Rayleigh dissipation potential that characterizes the energy dissipated as the fluid deforms is given by Arroyo and DeSimone (2009)

$$D[\mathbf{V}] = \eta \int_{\mathbb{R}^3} \mathbf{D} : \mathbf{D} dV, \tag{18}$$

where the rate of deformation tensor \mathbf{D} is the symmetric part of the velocity gradient $\nabla \mathbf{V}$. In addition, the fluid is subjected to a body force $\mathbf{b}(\mathbf{x})$. Finally, the fluid should satisfy $\nabla \cdot \mathbf{V} = 0$ since it is incompressible. Taking into account the incompressibility constraint by introducing a Lagrange multiplier, the Lagrangian for the system can be written as

$$\mathcal{L}[\mathbf{V}, p] = \eta \int_{\mathbb{R}^3} \mathbf{D} : \mathbf{D} dV - \int_{\mathbb{R}^3} p \nabla \cdot \mathbf{V} dV - \int_{\mathbb{R}^3} \mathbf{b} \cdot \mathbf{V} dV, \tag{19}$$

where the Lagrange multiplier p can be interpreted as the pressure in the fluid. Based on Onsager’s principle, the variational formulation for the dissipative system can be stated as (Arroyo et al., 2018),

$$[\mathbf{V}, p] = \arg \min_{\mathbf{w}} \arg \max_s \mathcal{L}[\mathbf{w}, s]. \tag{20}$$

Thus, taking variations with respect to \mathbf{V} and p yields the Euler–Lagrange equations for an incompressible Stokes fluid,

$$-\nabla p + \eta \nabla^2 \mathbf{V} + \mathbf{b} = 0, \quad \text{in } \mathbb{R}^3, \tag{21}$$

$$\nabla \cdot \mathbf{V} = 0, \quad \text{in } \mathbb{R}^3. \tag{22}$$

At the domain of the membrane, the body force \mathbf{b} in the above equation represents the force exerted by the membrane on the fluid as it deforms (or fluctuates). Thus, the body force is expressed as $\mathbf{b} = \mathbf{b}_t + b_n \mathbf{n}$, where \mathbf{n} denotes the unit normal vector at a

point on the surface of the membrane. \mathbf{b}_t is the tangential component of the body force exerted by the membrane whereas b_n arises from out-of-plane deformation of the membrane, and hence, is induced by its curvature elasticity and surface tension. In addition, as explained later in our statistical mechanics analysis, the body force may include a white noise that accounts for the thermal fluctuations as well as a force term associated with the active fluctuations of the membrane. The linearity of the Stokes equation enables a simple solution approach for inhomogeneous Eq. (21). Using a Green's function approach, the solution to the velocity field is derived as (Seifert, 1999; Turlier and Betz, 2018)

$$\mathbf{V}(\mathbf{x}) = \int_{\mathbb{R}^3} d^3\mathbf{x}' \mathcal{O}(\mathbf{x}, \mathbf{x}') \mathbf{b}(\mathbf{x}'), \tag{23}$$

where the Green's function $\mathcal{O}(\mathbf{x}, \mathbf{x}')$ is also called the Oseen tensor, and has the following expression (Doi and Edwards, 1986)

$$\mathcal{O}(\mathbf{x}, \mathbf{x}') = \Lambda(\mathbf{x} - \mathbf{x}') \left[\mathbf{I} + \frac{(\mathbf{x} - \mathbf{x}') \otimes (\mathbf{x} - \mathbf{x}')}{|\mathbf{x} - \mathbf{x}'|^2} \right], \tag{24}$$

with $\Lambda(\mathbf{x}) = 1/8\pi\eta|\mathbf{x}|$. Note that this solution is valid throughout the domain of the fluid. Assuming no-slip conditions at the membrane implies that the velocity of a fluid particle at the membrane should be equal to the velocity of the membrane at that point. That is,

$$\frac{\partial}{\partial t} \mathbf{R}(s_1, s_2) = \mathbf{V}(\mathbf{R}(s_1, s_2)). \tag{25}$$

Eq. (25) provides a description of the time evolution of the fluid membrane deforming in a viscous fluid. The normal component of Eq. (25) describes the shape change (or out-of-plane deformation) of the membrane as it relates the normal velocity of the membrane at a point to the normal variation of the membrane potential energy. For membranes without boundaries, the tangential variation of the membrane energy only has contribution from the surface tension and not from the bending energy since $\psi(H, K)$ does not explicitly depend on the parametrization coordinates (s_1, s_2) . Thus, the tangential component of Eq. (25) simply predicts a non-uniform surface tension which is needed to ensure local incompressibility which, in turn, is accommodated by flow of lipid molecules within the membrane (Seifert, 1999). Hence, it is neglected in most prior studies on passive and active membranes. We do note that tangential variations become relevant when boundary conditions, such as in the case of edges or pores, are considered (Steigmann et al., 2003; Arroyo and DeSimone, 2009; Biria et al., 2013) which are not pursued in the present study.

3.2. Fluctuations of passive membranes — dynamic analysis

Here, we apply the continuum theory developed above to study equilibrium thermal fluctuations of planar membranes as an illustrative example. The statistical mechanics results presented here are found in literature. We revisit the classical case of a passive membrane from Section 2 but consider it embedded in a viscous fluid. We consider a quasi-planar membrane and describe it using Monge representation as before. As discussed in the preceding section, the normal component of Eq. (25) yields the shape evolution of the membrane. In thermal equilibrium, b_n includes two terms — the normal component of the membrane elastic force and a random noise that accounts for collisions between the fluid particles and the membrane. In Monge gauge, the normal velocity of the membrane at a point is given by $\frac{\partial h}{\partial t}$. Then, substituting Eq. (23) in Eq. (25), the equation for the evolution of the membrane height $h(\mathbf{r}, t)$ is obtained as

$$\frac{\partial h}{\partial t}(\mathbf{r}, t) = \int_{\mathbb{R}^3} d\mathbf{r}' \Lambda(\mathbf{r} - \mathbf{r}') \left[-\frac{\delta F}{\delta h(\mathbf{r}, t)} + \xi^{\text{th}}(\mathbf{r}, t) \right], \tag{26}$$

where the variational derivative of the potential energy of the membrane in Eq. (12) with respect to the membrane height h is derived as

$$\frac{\delta F}{\delta h} = \kappa \Delta \Delta h - \sigma \Delta h, \tag{27}$$

and $\xi^{\text{th}}(\mathbf{r}, t)$ is a white noise with superscript “th” denoting the thermal origin of the noise. Substituting Eq. (27) and taking the spatial Fourier transform of Eq. (26), yields the well-known over-damped Langevin equation for a membrane (Cai and Lubensky, 1994, 1995; Prost and Bruinsma, 1996)

$$\frac{\partial h_{\mathbf{q}}(t)}{\partial t} = \Lambda_{\mathbf{q}} \left[-(\kappa q^4 + \sigma q^2) h_{\mathbf{q}}(t) + \xi_{\mathbf{q}}^{\text{th}}(t) \right], \tag{28}$$

where $\Lambda_{\mathbf{q}} = 1/4\pi\eta q$ and $q = |\mathbf{q}|$. According to the Langevin theory of Brownian motion, the thermal noise has zero mean and its auto-correlation function is defined such that the fluctuation–dissipation relation is satisfied (Sethna, 2006; Pathria and Beale, 2011; Turlier and Betz, 2018),

$$\langle \xi_{\mathbf{q}}^{\text{th}}(t) \xi_{\mathbf{q}'}^{\text{th}}(t') \rangle = \frac{2k_B T}{\Lambda_{\mathbf{q}}} \delta(\mathbf{q} - \mathbf{q}') \delta(t - t'). \tag{29}$$

We now define the temporal Fourier transform of $h_{\mathbf{q}}(t)$ using the relations

$$h_{\mathbf{q}}(\omega) = \int_{-\infty}^{\infty} h_{\mathbf{q}}(t) e^{-i\omega t} dt, \tag{30}$$

and

$$h_{\mathbf{q}}(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} h_{\mathbf{q}}(\omega) e^{i\omega t} d\omega. \tag{31}$$

Substituting the inverse Fourier transform Eq. (31) for $h_{\mathbf{q}}(t)$ in Eq. (28) we get,

$$i\omega h_{\mathbf{q}}(\omega) = -\Lambda_{\mathbf{q}}(\kappa q^4 + \sigma q^2)h_{\mathbf{q}}(\omega) + \Lambda_{\mathbf{q}}\xi_{\mathbf{q}}^{\text{th}}(\omega). \tag{32}$$

Here, we take the time derivative on the left hand side of Eq. (28) inside the integral since the inverse Fourier transform integral is over ω . On simplifying, we get

$$h_{\mathbf{q}}(\omega) = \frac{\Lambda_{\mathbf{q}}\xi_{\mathbf{q}}^{\text{th}}(\omega)}{\omega^2 + \omega_q^2} (-i\omega + \omega_q), \tag{33}$$

where ω_q is the membrane relaxation rate for mode \mathbf{q} given by

$$\omega_q = \Lambda_q(\kappa q^4 + \sigma q^2) = \frac{\kappa q^3 + \sigma q}{4\pi\eta}. \tag{34}$$

Taking the statistical average of $|h_{\mathbf{q}}(\omega)|^2$ in Fourier space and using the property of the thermal noise, the mode-dependent autocorrelation function is obtained as

$$\langle |h_{\mathbf{q}}(\omega)|^2 \rangle = \frac{2k_B T \Lambda_q}{\omega^2 + \omega_q^2}. \tag{35}$$

Finally, by integrating $\langle |h_{\mathbf{q}}(\omega)|^2 \rangle$ over all frequencies ω , the exact result for the equilibrium membrane fluctuations obtained from static analysis (Eq. (17)) is recovered. It seems interesting that even if we consider the membrane fluctuating in a viscous medium, the fluctuation spectra of a passive membrane fluctuating only under the influence of thermal noise does not depend on the viscosity of the embedding fluid. However, this is rather a consequence of how the auto-correlation for the thermal noise is defined in Eq. (29) so as to obey the fluctuation–dissipation theorem which provides a means of understanding how systems which are not in equilibrium finally achieve thermal equilibrium through exchange of energy between thermal fluctuations and dissipative mechanisms (Pathria and Beale, 2011).

3.3. Fluctuations of active membranes

Here, we demonstrate the application of the continuum theory developed above to study non-equilibrium (active) fluctuations of planar membranes. To this end, Eq. (28) is used as a starting point for modeling fluctuations of a quasi-planar membrane in a viscous medium subjected to thermal and active forces by appending the Langevin equation with additional forces due to active proteins and processes (Prost and Bruinsma, 1996; Manneville et al., 1999; Ramaswamy et al., 2000; Ramaswamy and Rao, 2001; Lin et al., 2006; Gov, 2004; Loubet et al., 2012). The active forces are incorporated in the body force through an additional noise term with active origins. Thus, the over-damped Langevin equation with active forces in the Fourier domain can be directly written as

$$\frac{\partial h_{\mathbf{q}}(t)}{\partial t} = -\Lambda_{\mathbf{q}}(\kappa q^4 + \sigma q^2)h_{\mathbf{q}}(t) + \Lambda_{\mathbf{q}}\xi_{\mathbf{q}}^{\text{th}}(t) + \Lambda_{\mathbf{q}}\xi_{\mathbf{q}}^{\text{a}}(t), \tag{36}$$

where $\xi_{\mathbf{q}}^{\text{a}}(t)$ is the Fourier transform of the active noise with the superscript “a” denoting the active origin of the force. In contrast to the thermal noise, the random active noise, $\xi_{\mathbf{q}}^{\text{a}}(t)$, is un-correlated in space but correlated exponentially in time. This is reasonable because if density fluctuations due to the diffusion of active proteins in the lipid bilayer are neglected, the active noise term can be assumed to be uncorrelated in space. In addition, active force due to an active protein switching between “on” and “off” states is expected to be exponentially correlated in time. Thus, the active force is assumed to have zero mean and the following form for the auto-correlation function (Turlier and Betz, 2018),

$$\langle \xi_{\mathbf{q}}^{\text{a}}(t)\xi_{\mathbf{q}'}^{\text{a}}(t') \rangle = \Gamma_q^{\text{a}} \delta(\mathbf{q} - \mathbf{q}') e^{-(t-t')/\tau^{\text{a}}}. \tag{37}$$

τ^{a} is a characteristic time-scale for the active process, and Γ_q^{a} is the amplitude of the active noise. The characteristics of the active noise source term need not be constrained *a priori* and will depend on the source of active forces, but it can be seen from Eq. (37) that when it is non-vanishing, equilibrium is no longer valid as the force does not satisfy the fluctuation–dissipation theorem. Based on experimental evidence, many prior studies on active membranes consider primarily two types of active forces — the so-called direct force and curvature force. Here, we summarize the approach taken in Lin et al. (2006) and Turlier and Betz (2018) to incorporate the specific nature of the active force into the model through Γ_q^{a} and τ^{a} , as described below.

- A direct force exerted by a protein is implemented as

$$\Gamma_q^{\text{a}} = \rho_a F_a^2, \tag{38}$$

where ρ_a is the density of active proteins and F_a is the normal force exerted by a protein. Forces induced by the cytoskeleton, such as in red blood cells, are represented as direct forces. Direct forces are also exerted by ion pumps such as in the case of

bacteriorhodopsin (BR), a light-activated proton pump embedded in the cell membranes of certain bacteria and Ca^{2+} -ATPase, a transmembrane protein that is known to change the bending rigidity of the membrane in the presence of ATP. We note that direct forces can either push the membrane up or down, and hence F_a can be positive, or negative (or zero when the active protein is in an “off” state). Since Γ_q^a is the amplitude of the auto-correlation function for the active noise, it is taken to depend on F_a^2 to make it independent of the sign or direction of the direct force. τ_a is the time constant with which the force turns on and off. For instance, the time period for the BR photocycle is 5 ms (Lin et al., 2006).

- A curvature force is implemented through the spontaneous curvature as

$$\Gamma_q^a = \rho_a(\kappa c_0 b^2 q^2)^2, \tag{39}$$

where c_0 is the positive or negative spontaneous curvature, b^2 is the area occupied by the active proteins, and κ is the bending modulus of the membrane. For an active protein, the spontaneous curvature induced by it could be c_0 or $-c_0$ or 0. Hence, the dependence of Γ_q^a is taken to be on c_0^2 to make it independent of the sign of the spontaneous curvature. Based on experiments, it has been shown that both BR and Ca^{2+} -ATPase proteins can induce local curvatures and affect the properties of the membrane during their activity (Lin et al., 2006).

As in the preceding section, we substitute the inverse Fourier transform in time of $h_q(t)$ in Eq. (36) to obtain an algebraic equation for $h_q(\omega)$ in Fourier space. Using the statistical correlations for the thermal noise (Eq. (29)) and active noise (Eq. (37)), the auto-correlation function for an active membrane is derived as,

$$\langle |h_q(\omega)|^2 \rangle = \frac{1}{\omega^2 + \omega_q^2} \left[2k_B T A_q + \frac{2\tau_a A_q^2 \Gamma_q^a}{1 + \omega^2 \tau_a^2} \right]. \tag{40}$$

The above result corresponds to steady state. Time correlation functions can also be derived for the given over-damped Langevin equation, Eq. (36). Eisenstecken et al. (2016) calculated the time correlations for relaxation dynamics of active filaments. Here, we are interested in steady state properties in the long time limit which are not influenced by initial conditions. Thus, integrating Eq. (40) over all the frequencies yields the steady-state fluctuation spectrum $\langle |h_q|^2 \rangle$ as

$$\langle |h_q|^2 \rangle = \frac{k_B T}{\kappa q^4 + \sigma q^2} + \frac{\Lambda_q^2 \Gamma_q^a}{\omega_q} \frac{1}{(\omega_q + 1/\tau_a)}. \tag{41}$$

Comparing Eqs. (17) and (41) reveals that the fluctuations of an active membrane are influenced not only by the active forces through parameters Γ_q^a and τ_a but also the viscosity of the embedding medium. Specifically, as demonstrated by the pioneering work of Prost and Bruinsma (1996) and following studies (Ramaswamy et al., 1999; Gov, 2004; Turlier and Betz, 2018), Eq. (41) shows that active forces indeed enhance the fluctuations of active membranes. This signature of active membranes provides a route to identifying active versus passive membranes by measuring their fluctuations through experiments.

Note that the fluctuation spectra for passive and active membranes given by Eqs. (17) and (41) respectively depend on the surface tension through the function ω_q . Since the surface tension is introduced as a Lagrange multiplier, it is determined self-consistently by using the areal constraint. Specifically, the change in surface area is calculated as (Turlier and Betz, 2018),

$$\Delta = \frac{A_{deformed} - A}{A} = \frac{1}{2A} \int_A d\mathbf{r} |\nabla h(\mathbf{r})|^2 \approx \frac{1}{2A} \sum_{\mathbf{q}} q^2 \langle |h(\mathbf{q})|^2 \rangle. \tag{42}$$

The above equation can be solved numerically to estimate the surface tension by using the relevant expression for $\langle |h(\mathbf{q})|^2 \rangle$ for passive or active membrane.

4. Fluctuations of active vesicles with nonlinear curvature elasticity

This section presents the statistical mechanics of closed membranes or vesicles in a viscous medium based on the continuum model developed in Section 3. Although results based on linear elasticity for active vesicles are found in the physics literature, a specific highlight of our work is that we extend the continuum theory to incorporate nonlinear curvature elasticity. Thus, this section contains the key results of this paper.

In Section 4.1, we first present the relevant mathematical expressions in curvilinear coordinates and apply the general framework of Section 3 to quasi-spherical vesicles. Next, we extend these calculations by incorporating nonlinear curvature elasticity and compare our results with the work of Ahmadpoor and Sharma (2016) under equilibrium considerations. In Section 4.2, we derive the results for the statistical mechanics of passive vesicles with nonlinear curvature elasticity. In Section 4.3, we derive the results for the statistical mechanics of active vesicles with nonlinear curvature elasticity subjected to active forces and compare our results with those reported by Loubet et al. (2012). Section 4.4 presents a discussion on how surface tension is incorporated in the study of the dynamics of membranes and its implications. In Section 4.5, we present numerical results for the fluctuation spectra for active vesicles and discuss the interplay of surface tension, active forces, and constitutive nonlinearity.

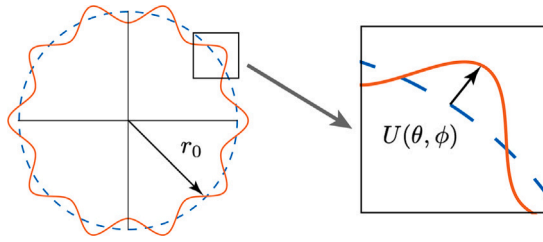


Fig. 3. Schematic of a fluctuating vesicle of mean radius r_0 . $U(\theta, \phi)$ denotes the perturbation normal to the surface.

4.1. General framework

Let \mathbb{S} be the surface of a fluctuating quasi-spherical vesicle with mean radius r_0 , defined as $\mathbb{S} := \{\mathbf{R} \in \mathbb{R}^3 : |\mathbf{R}| = r_0\}$. The membrane thickness is assumed to be d . Consider a small perturbation of the surface \mathbb{S} to \mathbb{S}_ϵ which takes a point on \mathbb{S} with position vector \mathbf{R} to \mathbf{R}_ϵ ,

$$\mathbf{R}_\epsilon = \mathbf{R} + \epsilon \mathbf{u}, \tag{43}$$

with

$$\mathbf{u} = \mathbf{u}_t + U \mathbf{n} = u_\alpha \mathbf{a}_\alpha + U \mathbf{n}, \quad \alpha = 1, 2, \tag{44}$$

where $U(\theta, \phi)$ is the normal variation (Fig. 3) and u_α are the tangential variations along directions \mathbf{a}_α in the tangent plane. Since there are no topological changes in the problem, we assume that the bending energy depends only on the mean curvature; that is, referring to Eq. (6), $\psi = \bar{\psi}(H)$. Then the variational derivative of the potential energy with respect to U is derived as (Biria et al., 2013)

$$F_U = \bar{\psi}_H (2H^2 - K) + \frac{1}{2} \Delta_S \bar{\psi}_H - 2H(\psi + \sigma). \tag{45}$$

In order to evaluate this expression, we first normalize U with respect to the mean radius r_0 by defining a dimensionless variable $u(\theta, \phi) = U(\theta, \phi)/r_0$. According to Helfrich (1986), H can be expanded in terms of u as follows,

$$H = \frac{1}{r_0} \left[-1 + u + \frac{1}{2} \underline{\Delta} u - u^2 - u \underline{\Delta} u \right], \tag{46}$$

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

$$\underline{\Delta} = \frac{1}{\sin \theta} \frac{\partial}{\partial \theta} \left(\sin \theta \frac{\partial}{\partial \theta} \right) + \frac{1}{\sin^2 \theta} \frac{\partial^2}{\partial \phi^2}. \tag{47}$$

Using Eq. (46), we can also obtain expressions for H^3 , and H^5 as follows where we have retained terms up to quadratic order. We will need these terms when we consider nonlinear curvature elasticity later.

$$H^3 = \frac{1}{r_0^3} \left[-1 + 3u + \frac{3}{2} \underline{\Delta} u - 6u^2 - 6u \underline{\Delta} u - \frac{3}{4} (\underline{\Delta} u)^2 \right], \tag{48}$$

$$H^5 = \frac{1}{r_0^5} \left[-1 + 5u + \frac{5}{2} \underline{\Delta} u - 15u^2 - 15u \underline{\Delta} u - \frac{5}{2} (\underline{\Delta} u)^2 \right]. \tag{49}$$

In contrast to the quadratic bending energy, we consider a form for $\bar{\psi}(H)$ that incorporates nonlinear curvature elasticity as

$$\bar{\psi}(H) = \frac{1}{2} \kappa (2H)^2 + \frac{1}{2} \gamma_c (2H)^4, \tag{50}$$

where we have ignored spontaneous curvature as before. We also assume the higher order elasticity modulus to be $\gamma_c = \kappa l_c^2$. The expression for γ_c shows that nonlinear considerations introduce a length-scale in the problem. Specifically, l_c denotes the critical length scale when the nonlinearity may be ignored and is typically assumed to be of the same order of magnitude as the thickness of the membrane. A detailed discussion on the choice of higher-order curvature elasticity terms and the physical underpinnings of l_c is provided by Ahmadpoor and Sharma (2016).

For convenience, we follow the convention prevalent in the biophysics community to write the bending energy in Eq. (50) as a function of $2H$ rather than H . Substituting Eq. (50) in Eq. (45), we get

$$F_U = 2\kappa \Delta_S H - 2H \sigma + 16\kappa l_c^2 (H^5 + \Delta_S H^3). \tag{51}$$

The first term arises from the (quadratic) Helfrich bending energy and the last term is a contribution from the quartic term. In deriving Eq. (51), we have used the relation $H^2 - K = 0$ in spherical coordinates (Schneider et al., 1984). Recall that the static equilibrium equation for a closed membrane is given by (Biria et al., 2013)

$$F_U = p, \tag{52}$$

where p is the osmotic pressure in the vesicle. For a spherical vesicle of radius r_0 , $H = -1/r_0$. Then, static equilibrium (in the absence of fluctuations) yields

$$2\sigma/r_0 - 16\kappa l_c^2/r_0^5 = p. \tag{53}$$

This is analogous to the Young–Laplace equation ($2\sigma/r_0 = p$) derived from linearized curvature elasticity which can also be recovered from the above equation by setting $l_c \rightarrow 0$. This shows that an effective surface tension can be defined in the case of nonlinear curvature elasticity which balances the osmotic pressure. To this end, observing that $\Delta_S H^3 = \frac{3}{r_0^3} \Delta_S H$, we can rewrite Eq. (51) as

$$F_U = 2\kappa(1 + 24 \frac{l_c^2}{r_0^2}) \Delta_S H - 2H (\sigma - 8\kappa l_c^2 H^4). \tag{54}$$

We can now define the effective surface tension as $\sigma_{eff} = \sigma - 8\kappa l_c^2 H^4$. Comparing Eq. (54) with the following expression for F_U for a quadratic energy,

$$F_U = 2\kappa \Delta_S H - 2H \sigma, \tag{55}$$

our results show how nonlinear curvature elasticity not only modifies the bending energy but also renormalizes the surface tension. We now expand the normalized virtual deformation u in spherical harmonics as

$$u(\theta, \phi) = \sum_{\ell \geq 0}^{\ell_{max}} \sum_{m=-\ell}^{\ell} u_{\ell,m} Y_{\ell,m}(\theta, \phi), \tag{56}$$

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

$$Y_{\ell,m}(\theta, \phi) = P_\ell^m(\theta) e^{im\phi}, \tag{57}$$

and satisfy the eigenvalue equation,

$$\Delta Y_{\ell,m} = -\ell(\ell + 1) Y_{\ell,m}. \tag{58}$$

We also define a dimensionless surface tension, $\bar{\sigma} = \sigma r_0^2 / \kappa$. Substituting these in the expressions given in Eqs. (46), (48), and (49), the variational derivative for the potential energy (Eq. (51)) is expressed as

$$F_U = -\frac{\kappa}{r_0^3} \sum_{\ell,m} \tilde{E}_\ell u_{\ell,m} Y_{\ell,m} - 2 \frac{\kappa}{r_0^3} \bar{\sigma}_{eff}, \tag{59}$$

where we use \tilde{E}_ℓ to denote the following expression,

$$\tilde{E}_\ell = [\ell^2(\ell + 1)^2 - 2\ell(\ell + 1)] (1 + 24 \frac{l_c^2}{r_0^2}) + \bar{\sigma}_{eff}(\ell + 2)(\ell - 1) \tag{60}$$

$$= (\ell + 2)(\ell - 1) \left[\ell(\ell + 1) \left(1 + 24 \frac{l_c^2}{r_0^2} \right) + \bar{\sigma}_{eff} \right], \tag{61}$$

and the dimensionless effective surface tension is defined as $\bar{\sigma}_{eff} = \bar{\sigma} - 8l_c^2 r_0^2 H^4$. Also, we write the summation as

$$\sum_{\ell,m} = \sum_{\ell \geq 2}^{\ell_{max}} \sum_{m=-\ell}^{\ell}, \tag{62}$$

where the summation starts at $\ell = 2$ since $\ell = 0$ corresponds to change in sphere size and $\ell = 1$ corresponds to translation (Helfrich, 1986). If we consider only the Helfrich bending energy, the expression for F_U in Eq. (55) reduces to

$$F_U = 2 \frac{\kappa}{r_0^3} [-\Delta u - \frac{1}{2} \Delta \Delta u] - 2 \frac{\kappa}{r_0^3} \bar{\sigma} [1 - u - \frac{1}{2} \Delta u] \tag{63}$$

$$= -\frac{\kappa}{r_0^3} \sum_{\ell,m} [\ell^2(\ell + 1)^2 - 2\ell(\ell + 1) + \bar{\sigma}(\ell + 2)(\ell - 1)] u_{\ell,m} Y_{\ell,m} - 2 \frac{\kappa}{r_0^3} \bar{\sigma} \tag{64}$$

$$= -\frac{\kappa}{r_0^3} \sum_{\ell,m} (\ell + 2)(\ell - 1) [\ell(\ell + 1) + \bar{\sigma}] u_{\ell,m} Y_{\ell,m} - 2 \frac{\kappa}{r_0^3} \bar{\sigma} \tag{65}$$

$$= -\frac{\kappa}{r_0^3} \sum_{\ell,m} E_\ell u_{\ell,m} Y_{\ell,m} - 2 \frac{\kappa}{r_0^3} \bar{\sigma}. \tag{66}$$

Thus, for quadratic energy, we recover the results available in existing literature ((Helfrich, 1986; Milner and Safran, 1987; Seifert, 1999; Gov, 2004) to cite a few). The only difference in Eqs. (59) and (66) is the use of E_ℓ (and $\bar{\sigma}$) in Eq. (66) and \tilde{E}_ℓ (and $\bar{\sigma}_{eff}$) in Eq. (59). If we take $l_c \rightarrow 0$, \tilde{E}_ℓ reduces to E_ℓ . We also emphasize that, in deriving Eq. (59), we have retained only terms for H^5 and $\Delta_S H^3$ that are linear in u and Δu so that

$$H^5 + \Delta_S H^3 = 1 - \frac{11}{2} \Delta u - 5u - \frac{3}{2} \Delta \Delta u. \tag{67}$$

This is consistent with prior studies such as by Helfrich (1986) and Seifert (1999) which also retain only linear order terms in Eq. (46) to obtain Eq. (63) in terms of u and \underline{u} . It is interesting that even while keeping only linear order terms from Eq. (46), (48), and (49), there is a contribution from nonlinear elasticity in the expression for F_U in Eq. (59). Thus, the form for F_U in Eqs. (59) and (66) remains the same in the case of linear or nonlinear curvature elasticity when only linear terms are retained. Only the coefficient E_ℓ is renormalized to \tilde{E}_ℓ .

4.2. Passive fluctuations under nonlinear curvature elasticity

Here, we develop the statistical mechanics of vesicles with nonlinear curvature elasticity fluctuating in a viscous medium. To this end, we follow the prior work on passive vesicles using linear elasticity and extend the approach to incorporate constitutive nonlinearity.

As shown for quasi-planar membranes in Section 3, using F_U in the normal body force b_n in Eq. (23), we can follow the Green’s function approach to derive the over-damped Langevin equation. However, solving the Stokes equation using the Oseen tensor with spherical harmonics is tedious (Lomholt, 2006). In order to obtain analytical results for a quasi-spherical vesicle embedded in a three-dimensional fluid, prior studies on the dynamics of vesicles have adopted the classical Lamb’s solution to derive the Langevin equation (Schneider et al., 1984; Milner and Safran, 1987; Seifert, 1999; Lomholt, 2006). We follow the same approach and refer the reader to these papers for a detailed presentation of the calculations.

The Lamb’s solution is a classical result in hydrodynamics literature for fluids with low Reynolds number (Lamb, 1932; Happel and Brenner, 1983). It describes the Stokes flow around a sphere and yields analytical results for the velocity field and pressure in a Stokes fluid in the presence of a sphere. In the context of quasi-spherical vesicles, the Lamb’s solution provides a more straightforward approach to analytically obtain the velocity and stress field on the surface of the quasi-spherical vesicle and obtain the over-damped Langevin equation using spherical harmonics compared to the Green’s function approach employed for quasi-planar membranes. A key approximation required to apply the Lamb’s solution for quasi-spherical vesicles is to assume that all stresses act on a sphere of mean radius r_0 rather than on the time-dependent vesicle configuration (Seifert, 1999). The solution is used to determine the stress in the fluid at the vesicle surface. Traction boundary conditions are enforced to equate the normal and shear stress in the fluid at the membrane surface with the normal and shear stress in the membrane respectively. The normal component then yields the over-damped Langevin equation in terms of $u_{\ell,m}$.

Since nonlinear bending energy retains the same general form for F_U in spherical coordinates as the conventional quadratic energy, the expression for the over-damped Langevin equation for a passive membrane with nonlinear curvature elasticity remains the same as found in literature and is given by

$$\frac{\eta}{\Gamma_\ell} \dot{u}_{\ell,m}(t) = -\frac{\kappa}{r_0^3} \tilde{E}_\ell u_{\ell,m} + \xi_{\ell,m}^{th}(t), \tag{68}$$

where E_ℓ for a quadratic energy is now replaced with \tilde{E}_ℓ when a quartic energy term is included and Γ_ℓ is given by (Seifert, 1999)

$$\Gamma_\ell = \frac{\ell(\ell+1)}{4\ell^3 + 6\ell^2 - 1}. \tag{69}$$

In the derivation of Eq. (68), we have used the fact that the surface tension term $2\kappa\bar{\sigma}/r_0^3$ in Eq. (66) balances the osmotic pressure p . This is the zeroth order equation obtained when the membrane force is equated to the Lamb’s solution and is not explicitly written in the literature on dynamics of vesicles.

The last term in Eq. (68) is the thermal (white) noise and is uncorrelated in space and time. The mean and the auto-correlation function of the thermal noise can be expressed as

$$\langle \xi_{\ell,m}^{th}(t) \rangle = 0, \tag{70}$$

$$\langle \xi_{\ell,m}^{th}(t) \xi_{\ell',m'}^{th}(t')^* \rangle = 2\eta \frac{k_B T}{r_0^3} \frac{1}{\Gamma_\ell} \delta_{\ell\ell'} \delta_{mm'} \delta(t-t'). \tag{71}$$

Taking Fourier transform of Eq. (68) in time, we get an expression for $u_{\ell,m}(\omega)$. We can then calculate the statistical average, $\langle |u_{\ell,m}(\omega)|^2 \rangle$ using the properties of the thermal noise. Integrating the result over all frequencies, we determine the fluctuation spectra for a quasi-spherical vesicle with nonlinear curvature elasticity as

$$\langle u_{\ell,m}(t) u_{\ell,m}^*(t) \rangle = \frac{k_B T}{\kappa \tilde{E}_\ell}. \tag{72}$$

As expected, the form of the fluctuation spectrum with nonlinear curvature elasticity is same as that reported in literature for quadratic energy (Schneider et al., 1984; Milner and Safran, 1987; Seifert, 1999; Lomholt, 2006) with \tilde{E}_ℓ replacing E_ℓ . Furthermore, the above result for a passive vesicle has also been derived previously for quadratic energy using equilibrium statistical mechanics (Helfrich, 1986). As seen for quasi-planar membranes, the fluctuation spectrum for a passive vesicle is independent of the viscosity of the medium owing to the fluctuation–dissipation theorem.

4.3. Active fluctuations under nonlinear curvature elasticity

Here, we develop the statistical mechanics of active vesicles with nonlinear curvature elasticity following the approach of Section 4.2. To this end, we append the over-damped Langevin equation derived in the previous section (Eq. (68)) with a random force term accounting for active forces,

$$\frac{\eta}{\Gamma_\ell} \dot{u}_{\ell,m}(t) = -\frac{\kappa}{r_0^3} \tilde{E}_\ell u_{\ell,m} + \xi_{\ell,m}^{th}(t) + \xi_{\ell,m}^a(t), \tag{73}$$

where $\xi_{\ell,m}^a(t)$ denotes the noise from active processes. We will use the form for the active force proposed by Loubet et al. (2012) for spherical vesicles. This active noise has the following properties:

$$\langle \xi_{\ell,m}^a(t) \rangle = 0 \tag{74}$$

$$\langle \xi_{\ell,m}^a(0) \xi_{\ell,m}^a(t)^* \rangle = \chi_a x_\ell \delta_{\ell\ell'} \delta_{mm'} \exp\left[-\frac{|t|}{\tau_a}\right], \tag{75}$$

where τ_a is a characteristic correlation time of the active process studied. χ_a is the strength of the noise and has the dimension of a force per unit area squared. x_ℓ is a dimensionless quantity that carries the wave number dependency of the noise. By spherical symmetry, it can only be a function of ℓ . Note that the form for the auto-correlation function for $\xi_{\ell,m}^a(t)$ (Eq. (75)) is qualitatively similar to that for $\xi_q^a(t)$ (Eq. (37)). Here we use the form proposed by Loubet et al. (2012) in order to easily compare our results with their results. Assuming steady state, the fluctuation spectrum can be determined from the over-damped Langevin equation (Eq. (73)) and the properties of the thermal and active noise as

$$\langle |u_{\ell m}|^2 \rangle = \frac{k_B T}{\kappa \tilde{E}_\ell} \left(1 + \tilde{\chi}_a \frac{x_\ell}{\tilde{E}_\ell} \frac{\tau_a}{\tau_a + t_m^\ell} \right), \tag{76}$$

where

$$\tilde{\chi}_a = \chi_a \frac{r_0^6}{k_B T \kappa}, \quad t_m^\ell = \frac{\eta r_0^3}{\kappa \Gamma_\ell \tilde{E}_\ell}. \tag{77}$$

$\tilde{\chi}_a$ is a non-dimensional measure for the strength of the noise, and t_m^ℓ is the correlation time for mode ℓ of the shape in thermal equilibrium. It is interesting to note that the formal expression for the fluctuation spectrum with nonlinear bending energy is the same as that for the fluctuation spectrum with quadratic bending energy with \tilde{E}_ℓ replacing E_ℓ . Furthermore, these results reveal that there is an additional contribution to the fluctuations of active membranes proportional to the strength of the noise $\tilde{\chi}_a$. In other words, active forces tend to modify the fluctuations of vesicles as observed in experiments.

4.4. Surface tension in fluctuating quasi-spherical vesicles

We note that there is a lot of variation and discussion in the literature on incorporating surface tension, especially in the case of dynamics of quasi-spherical vesicles (Schneider et al., 1984; Milner and Safran, 1987; Seifert, 1995; Cai and Lubensky, 1995; Seifert, 1999; Miao et al., 2002). Here we present a systematic explanation that we hope can provide a context for the seemingly disparate treatment of surface tension. It also serves to clarify when the tangential component of Eq. (25) is considered and when it is ignored in the study of dynamics of spherical vesicles.

As a Lagrange multiplier, the surface tension has to be determined from the change in area of the quasi-spherical vesicle, as shown for planar membranes in Section 3. Following Eq. (42), the change in area of the quasi-spherical vesicle is given by (Helfrich, 1986; Loubet et al., 2012)

$$\Delta = \sum_{\ell,m} \langle |u_{\ell m}|^2 \rangle \frac{(\ell+2)(\ell-1)}{2} \tag{78}$$

$$= \sum_{\ell} \sum_{m=-\ell}^{\ell} \langle |u_{\ell m}|^2 \rangle \frac{(\ell+2)(\ell-1)}{2} = \sum_{\ell} \langle |u_{\ell m}|^2 \rangle \frac{(\ell+2)(\ell-1)(2\ell+1)}{2}, \tag{79}$$

where we have used the fact that $\langle |u_{\ell m}|^2 \rangle$ is independent of m . Thus, substituting the expression for fluctuation spectrum in the above equation we can get the expressions for the area change for a passive membrane with nonlinear curvature elasticity as

$$2\Delta \frac{\kappa}{k_B T} = \sum_{\ell} \frac{(\ell+2)(\ell-1)(2\ell+1)}{\tilde{E}_\ell}, \tag{80}$$

and for an active membrane with nonlinear curvature elasticity as

$$2\Delta \frac{\kappa}{k_B T} = \sum_{\ell} \frac{(\ell+2)(\ell-1)(2\ell+1)}{\tilde{E}_\ell} \left(1 + \tilde{\chi}_a \frac{x_\ell}{\tilde{E}_\ell} \frac{\tau_a}{\tau_a + t_m^\ell} \right). \tag{81}$$

These equations have to be solved numerically to estimate the surface tension in a passive or active membrane. Note that Eqs. (80) and (81) can be easily reduced to the case of linearized curvature elasticity by replacing \tilde{E}_ℓ with E_ℓ . We refer the reader to the

papers by Seifert and coworkers for an elaborate discussion on the surface tension estimates based on different limiting cases in passive (Seifert, 1995) and active (Loubet et al., 2012) membranes.

If we consider the fluctuations of a quasi-spherical vesicle as mere perturbations about a mean spherical shape of radius r_0 , it is reasonable to assume that the surface tension is defined on the sphere. For a spherical vesicle, we recall that the surface tension σ balances the difference between the internal and external pressure. If we denote the pressure difference across the vesicle surface as p , this force balance based on linearized curvature elasticity can be written in the form of the well-known Young–Laplace equation, $2\sigma/r_0 = p$. Thus, the surface tension is indeed uniform over the sphere and can be determined from the areal constraint as shown above. Now if we consider the tangential variation of the potential energy (Eq. (7)), the variational derivative is given by (Steigmann et al., 2003; Biria et al., 2013)

$$F_\alpha = -\frac{\partial\psi}{u_\alpha} - \frac{\partial\sigma}{u_\alpha}, \quad \alpha = 1, 2, \tag{82}$$

where u_α are the tangential variations (Eq. (44)). Since $\psi(H)$ does not explicitly depend on the tangential coordinates and σ is a constant on the sphere surface, the above equation shows that the tangential force exerted by the membrane is zero. Thus, the tangential force balance on the sphere does not yield new information, and it is reasonable to ignore the tangential component of Eq. (25) for dynamics of both passive and active vesicles.

Some studies adopt a more rigorous approach for incorporating surface tension and consider the fluctuations of the surface tension in a quasi-spherical vesicle (Schneider et al., 1984; Seifert, 1999; Miao et al., 2002). Here, σ is expanded in spherical harmonics along with the normal variation U as,

$$\sigma(\theta, \phi) = \sigma_0 + \sum_{\ell \geq 2}^{\ell_{\max}} \sum_{m=-\ell}^{\ell} \sigma_{\ell,m} Y_{\ell,m}(\theta, \phi), \tag{83}$$

where σ_0 corresponds to the zeroth order surface tension which is defined on the mean sphere. The higher order modes of σ are non-uniform on the membrane surface. Following the preceding discussion, σ_0 balances the pressure difference across the membrane surface and can be determined from the areal constraint. The higher modes, being non-uniform, are determined by equating the tangential stress due to the membrane given by Eq. (82) to the tangential stress from the Lamb’s solution and solving for $\sigma_{\ell,m}$. Thus, if we consider only the zeroth order term and ignore the higher order terms, it suffices to consider the normal component of Eq. (25) which yields the over-damped Langevin equation for membrane shape evolution and estimate the surface tension self-consistently from the areal constraint. Physically, this is a reasonable assumption, especially for small vesicles as they cannot accommodate inhomogeneities on membrane surface and hence surface tension can be assumed to be a constant. In the present study, we have followed this assumption and work only with the zeroth order term for surface tension.

4.5. Computation of fluctuation spectra of active vesicles

Here, we present the fluctuation spectra of active vesicles based on the model developed in the preceding section for some special cases of active forces. We will focus on small size vesicles where the effect of nonlinear curvature elasticity is known to be accentuated. In order to evaluate Eq. (76) numerically and understand the interplay of nonlinear curvature elasticity and activity, we investigated the limiting case of very long correlation time for the active processes relative to the correlation time t_m^ℓ of mode ℓ . Thus, for $\tau_a \gg t_m^\ell$, Eq. (76) reduces to

$$\langle |u_{\ell m}|^2 \rangle = \frac{k_B T}{\kappa \tilde{E}_\ell} \left(1 + \tilde{\chi}_a \frac{x_\ell}{\tilde{E}_\ell} \right). \tag{84}$$

As in the case of quasi-planar active membranes, we can consider two types of active forces — direct force and curvature force. Consistent with the expressions for active forces provided in Eqs. (38) and (39), we used $x_\ell = 1$ for direct force, and $x_\ell = (\ell + 2)^2(\ell - 1)^2/4$ for curvature force (Loubet et al., 2012). For each type of active force, we present our results with and without surface tension. Since most prior work on active membranes has focused on very large vesicles ($r_0 \sim 1000d$), we first compared our results for nonlinear curvature elasticity with the results by Loubet et al. (2012). We found that nonlinear curvature elasticity has negligible effect on the fluctuations of such large vesicles. We then considered the case of very small vesicles with $r_0 = 5d$. We chose this extremely small size as a limiting case since (Ahmadpoor and Sharma, 2016) show that nonlinear curvature elasticity dramatically increases the bending modulus at these vesicle sizes. We then computed the surface tension in the absence and presence of activity using Eq. (80) and (81) respectively. The values for different cases are reported in the figure caption. Using the appropriate value of surface tension, we computed the fluctuation spectrum for the different types of active forces. The results are plotted in Fig. 4 for direct force and Fig. 5 for curvature force.

We make several observations. First, surface tension plays a dominant role in the fluctuations. Specifically, the fluctuations are dramatically reduced by orders of magnitude for tense membranes when compared to tensionless membranes as seen by comparing (a) and (b) cases for direct and curvature force results. Furthermore, Figs. 4(a) and 5(a) show that activity substantially increases the fluctuations in the absence of surface tension. Second, we find a rather unexpected interplay of activity and surface tension. For the direct force case, when surface tension is considered (Fig. 4(b)), activity increases the fluctuations at lower modes but decreases the fluctuations at higher modes. In contrast, for the curvature force case, when surface tension is considered (Fig. 5(b)), activity decreases the fluctuations at lower modes but increases the fluctuations at higher modes. The physical underpinning of this role of activity, especially in small vesicles, is not yet clear to us. In fact, we do not find this interplay for large vesicles in which case

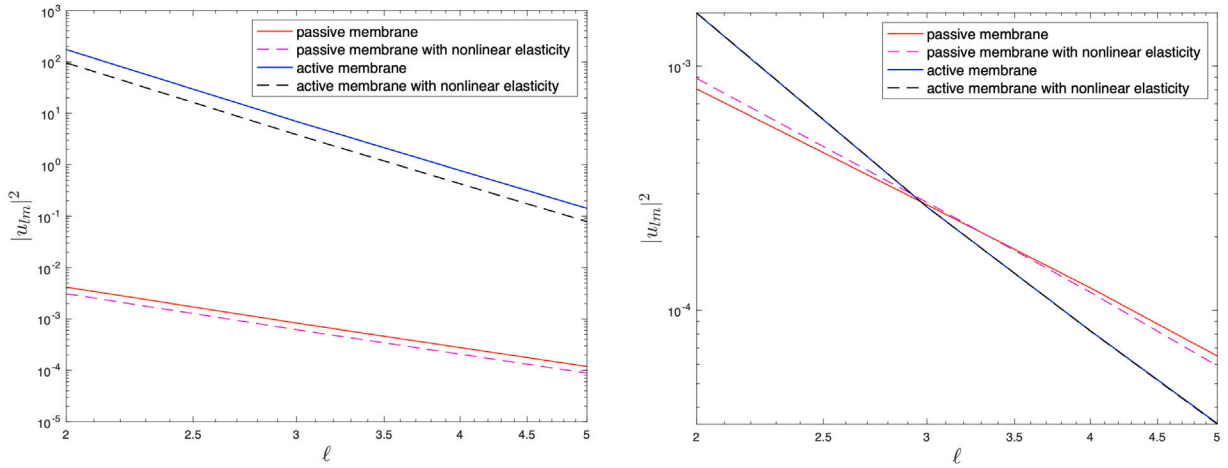


Fig. 4. Plots for $\langle |u_{\ell m}|^2 \rangle$ versus ℓ for active vesicles with nonlinear curvature elasticity for the case of direct force (a) with $\bar{\sigma} = 0$ and (b) with surface tension. We use the following parameters: $\kappa = 10k_B T$, $r_0 = 5d$, $l_{max} = r_0/d$, $l_c = 3 \times 10^{-9}$ m, and $d = 5 \times 10^{-9}$ m consistent with prior study by Ahmadpoor and Sharma (2016) for incorporating nonlinear curvature elasticity. For these parameters, we obtain $\bar{\sigma}^{th} = 25$ and $\bar{\sigma}_{nl}^{th} = 20$ where “nl” denotes the nonlinear elasticity case. For the active force, we use $x_\ell = 1$ and $\tilde{\chi}_a = 10^6$ based on a prior study by Loubet et al. (2012). To estimate the surface tension, we use $\Delta/4\pi = 0.003$ for the excess area. For these parameters for active force, we obtain $\bar{\sigma}^a = 1950$ and $\bar{\sigma}_{nl}^a = 1945$.

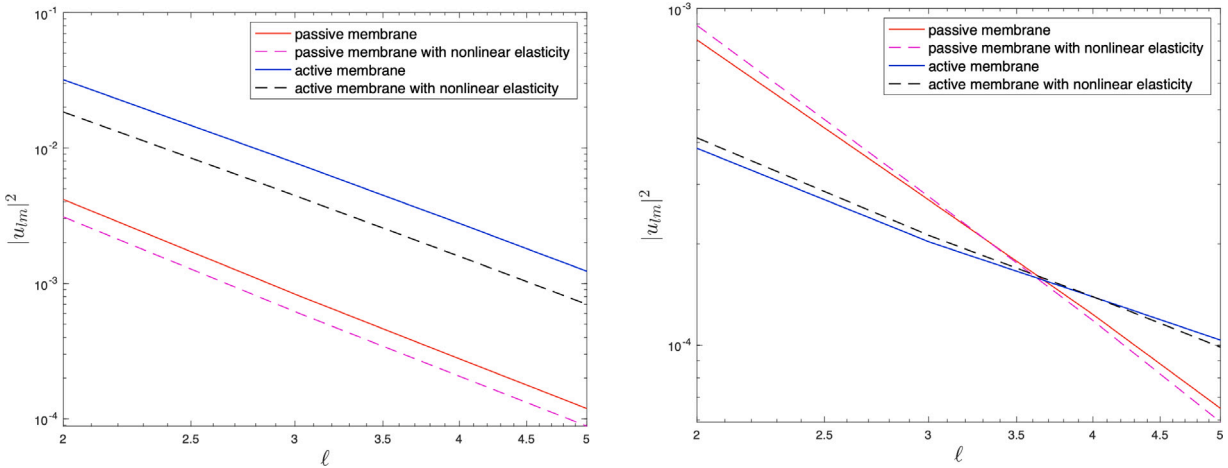


Fig. 5. Plots for $\langle |u_{\ell m}|^2 \rangle$ versus ℓ for active vesicles with nonlinear curvature elasticity for the case of curvature force (a) with $\bar{\sigma} = 0$ and (b) with surface tension. We use the following parameters: $\kappa = 10k_B T$, $r_0 = 5d$, $l_{max} = r_0/d$, $l_c = 3 \times 10^{-9}$ m, and $d = 5 \times 10^{-9}$ m consistent with prior study by Ahmadpoor and Sharma (2016) for incorporating nonlinear curvature elasticity. For these parameters, we obtain $\bar{\sigma}^{th} = 25$ and $\bar{\sigma}_{nl}^{th} = 20$ where “nl” denotes the nonlinear elasticity case. For the active force, we use $x_\ell = (\ell + 2)^2(\ell - 1)^2/4$ and $\tilde{\chi}_a = 40$ and to estimate the surface tension, we use $\Delta/4\pi = 0.003$ for the excess area. For these parameters for active force, we obtain $\bar{\sigma}^a = 87$ and $\bar{\sigma}_{nl}^a = 80$.

activity enhances the fluctuations for lower modes and does not impact higher modes. This is also seen in the results of Loubet et al. (2012) for large vesicles under direct or curvature force. Third, although nonlinear curvature elasticity tends to decrease the fluctuations in the presence or absence of activity, it does not seem to have a significant impact on fluctuations even for extremely small vesicles without surface tension. Thus, based on our numerical results, surface tension, followed by activity are the dominant players in the fluctuations of active vesicles.

5. Vesicle size distribution of active vesicles

In order to understand the biophysical implications of active forces and fluctuations in vesicles, we revisit the problem of determining vesicle size distribution (Helfrich, 1986; Kleinert, 1986; Ahmadpoor and Sharma, 2016). Most prior studies in biophysics literature have modeled the contribution of active forces only in the fluctuations of membranes. These studies provided unprecedented insights by facilitating comparison with experimental data on fluctuations and elucidating the role of active forces in enhancing the fluctuations. We seek to go beyond these studies and investigate the role of active fluctuations in a biophysical problem of interest. Specifically, we study vesicle size distribution as an illustrative example.

We note that the results presented in this section are based on the assumption that the vesicles, even under the influence of active forces, are in thermal equilibrium. The validity of this assumption warrants a deeper theoretical investigation and calibration through experiments. However, since we do not yet have non-equilibrium statistical mechanics tools at our disposal to model active matter far from equilibrium, we rely on the assumption that active vesicles are close to equilibrium. This assumption permits us to employ equilibrium statistical mechanics to estimate the vesicle size distribution from the steady-state fluctuation spectra derived in the preceding section. In other words, we assume that the fluctuation spectra obtained under the influence of active forces, are essentially associated with equilibrium thermal fluctuations. Such an assumption has been employed in prior studies. Often, by assuming that the system is near equilibrium, non-equilibrium effects are considered by way of renormalizing the temperature (which is an equilibrium notion) and assuming the system to be in thermal equilibrium at that renormalized temperature. In fact, active fluctuations are identified in experimental studies in the form of an “effective temperature” (Turlier and Betz, 2018). Here, instead of defining an effective temperature, we define an effective free energy (a notion rooted in equilibrium statistical mechanics).

5.1. Analytical results

We first present the derivation of the expression for vesicle size distribution based on the fluctuation spectrum of active vesicles following the works of Helfrich (1986) and Ahmadpoor and Sharma (2016).

Assuming our active vesicle to be in equilibrium, we define a free energy, F . According to Helfrich (1986), the vesicle distribution can then be described by the probability distribution function,

$$w(N) = A \exp(-F/k_B T), \tag{85}$$

where N is the number of amphiphilic molecules comprising the vesicle. The proportionality constant A is determined by normalizing the probability distribution function using the constraint that

$$\int_0^\infty w(N) dN = 1. \tag{86}$$

The total free energy for a spherical vesicle with N molecules and chemical potential μ is given by (Helfrich, 1986)

$$F_0 = \mu N + 2\pi\kappa, \tag{87}$$

where the bending energy of a sphere based on linear elasticity is $2\pi\kappa$. According to Ahmadpoor and Sharma (2016), the free energy, \tilde{F} of a fluctuating spherical vesicle can be estimated from the fluctuation spectra as

$$\tilde{F} = \frac{k_B T}{2} \sum_{\ell, m} \log G_{\ell m}, \tag{88}$$

where $G_{\ell m}$ can be obtained from the relation

$$\langle |u_{\ell m}|^2 \rangle \approx \frac{k_B T}{G_{\ell m}}. \tag{89}$$

To take into account the effect of activity and nonlinear curvature elasticity, we compare Eqs. (84) and (89) to get the expression for $G_{\ell m}$ for the case of an active vesicle as

$$G_{\ell m} = \kappa \frac{\tilde{E}_\ell}{1 + \tilde{f}_\ell}, \text{ where } \tilde{f}_\ell = \frac{\tilde{\chi}_a x_\ell}{\tilde{E}_\ell}. \tag{90}$$

To enable comparison with the results of Ahmadpoor and Sharma (2016), we rewrite \tilde{E}_ℓ in terms of the eigenvalues defined in Eq. (58) as

$$\kappa \tilde{E}_\ell = \kappa \left[\ell^2(\ell + 1)^2 - 2\ell(\ell + 1) \right] \left(1 + 24 \frac{l_c^2}{r_0^2} \right) + \kappa \bar{\sigma}_{eff}(\ell + 2)(\ell - 1) = c_1 q^4 - c_2 q^2 + c_3 q^2, \tag{91}$$

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

$$c_1 = c_2 = \kappa \left(1 + 24 \frac{l_c^2}{r_0^2} \right), \quad c_3 = \kappa \bar{\sigma}_{eff}. \tag{92}$$

Then, following the work of Ahmadpoor and Sharma (2016), we get the total free energy of the fluctuating active vesicle with N molecules as

$$F = \mu N + 2\pi \left(c_1 - \frac{k_B T}{4\pi} \frac{c_2}{c_1} \log N + \frac{c_3}{c_1} \frac{k_B T}{4\pi} \log N - \frac{k_B T}{4\pi} \frac{\kappa \tilde{\chi}_a}{4c_1} N \right), \tag{93}$$

where we have executed the summations by converting them into integrals as shown below.

$$\sum_{\ell, m} \frac{1}{q^2} = \sum_{\ell \geq 2} \sum_{m=-\ell}^{\ell} \frac{1}{q^2} = \int_{\ell_{min}}^{\ell_{max}} \frac{2\ell + 1}{\ell(\ell + 1)} d\ell = \log N, \tag{94}$$

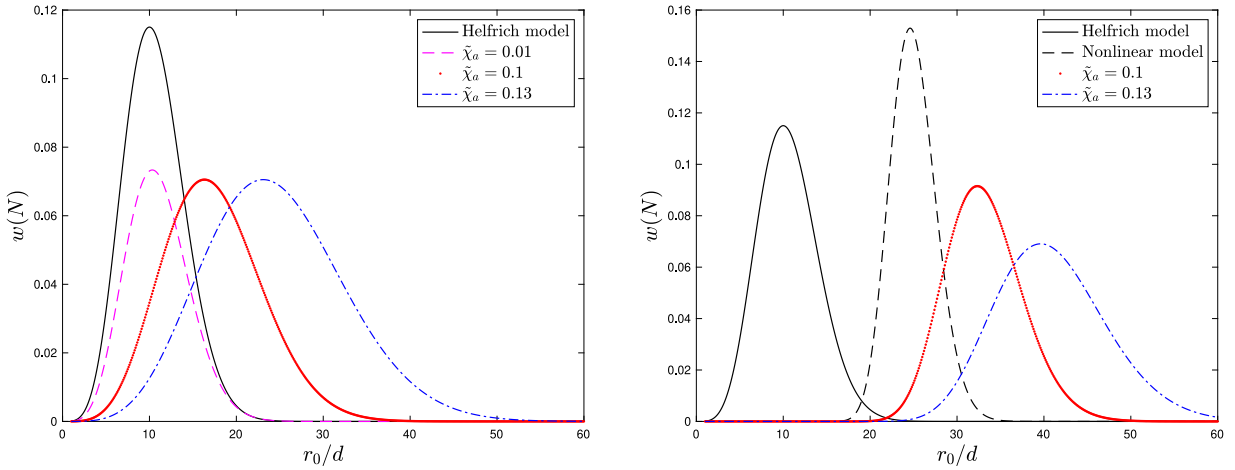


Fig. 6. Plots for probability density for vesicle size distribution as a function of r_0/d in the case of (a) linearized curvature elasticity and (b) nonlinear curvature elasticity in the presence of activity with strength $\tilde{\chi}_a$ ranging from 0 to 0.13. We use the following parameters: $\kappa = 10k_B T$, $l_c = 3 \times 10^{-9}$ m, and $d = 5 \times 10^{-9}$ m.

where we define $q^2 = \ell(\ell + 1)$ and note that $\sum_m = 2\ell + 1$ since the term inside the summation in Eq. (94) is independent of m . The value of ℓ_{max} can be determined from the total number of modes or degrees of freedom, N as

$$N = \sum_{\mathbf{q}} = \sum_{\ell} \sum_{m=-\ell}^{\ell} 1 = \sum_{\ell} (2\ell + 1) = \frac{4\pi r_0^2}{A_0}, \tag{95}$$

where A_0 is the area per amphiphilic molecule and is taken to be about d^2 , d being the thickness of the membrane. Using $x_{\ell} = (\ell + 2)^2(\ell - 1)^2/4$ for curvature force, the active term is approximated as

$$\sum_{\ell, m} \kappa \frac{\tilde{\chi}_a x_{\ell}}{\bar{E}_{\ell}} \approx \frac{\kappa \tilde{\chi}_a}{4c_1} N. \tag{96}$$

Substituting Eq. (93) in Eq. (85), we get the probability density in the form

$$w(N) = A N^{a'/2} \exp[-f(N)], \tag{97}$$

where we have used

$$a' = \frac{c_2}{c_1} - \frac{c_3}{c_1}, \tag{98}$$

and

$$f(N) = \frac{1}{k_B T} (A_1 N + \frac{A_2}{N}). \tag{99}$$

Here, we have also used the relation $r_0^2 = N A_0 / 4\pi$ in the expression for c_1 in Eq. (93) with

$$A_1 = \mu - \frac{\kappa \tilde{\chi}_a}{8c_1}, \quad A_2 = \frac{172\kappa\pi^2}{k_B T} \frac{l_c^2}{d^2}. \tag{100}$$

5.2. Numerical results

Here, we present numerical results for vesicle size distribution of active vesicles based on Eq. (97). The results for the probability of finding vesicles of a certain radius are plotted as a function of the normalized radius r_0/d in Fig. 6 for varying degrees of strength with $\tilde{\chi}_a$ ranging from 0 to 0.13. For our numerical calculations presented below, we assume $\bar{\sigma}_{eff} = 0$. The solid line in Figs. 6 (a) and (b) shows the results from Helfrich’s work based on linear elasticity (Helfrich, 1986). The dashed line in Fig. 6(b) shows the distribution for passive vesicles including nonlinear curvature elasticity derived based on our expressions of c_1 , c_2 and c_3 . All other plots show how the distribution of active vesicles can change depending on the strength of the active forces. Consistent with the findings of Ahmadpoor and Sharma (2016), we observe that nonlinear curvature elasticity introduces a cut-off radius as shown in Fig. 6(b) which is realistically expected and supported by experimental evidence (Xu et al., 2013).

The plots shows that the presence of active forces results in a notable shift in the probability density function towards larger vesicle sizes when compared to corresponding results for passive vesicles with linear (Fig. 6(a)) and nonlinear curvature elasticity (Fig. 6(b)). This implies that active vesicles can attain size distributions that are different from passive vesicles. In other words, by tuning the active forces, one can, in principle, go beyond vesicle size distribution predicted solely based on thermal fluctuations

using linearized or nonlinear curvature elasticity. It is indeed a tantalizing speculation that active forces can enable active vesicles to attain sizes that are deemed improbable for passive vesicles. Nevertheless, we note that a more accurate quantification of the effect of active forces and the resulting size distribution would require non-equilibrium statistical mechanics approaches since our numerical results presented in Fig. 6 are based on the assumption that active vesicles are near equilibrium.

The expressions for A_1 and A_2 in Eq. (100) show that the effect of the curvature-mediated force varies as N . Repeating the same calculations for direct force with $x_c = 1$ shows that its effect varies as $1/N$, and hence, does not have much impact on the vesicle size distribution. Thus, our results indicate that vesicle size is determined more strongly by forces that induce curvature rather than direct forces which seems physically reasonable.

We wish to make a comment on the parameters c_1 , c_2 and c_3 obtained in our work for nonlinear curvature elasticity. If we take $l_c \rightarrow 0$, we recover the results for quadratic energy. However, the coefficients of l_c^2/r_0^2 are different from those reported by Ahmadpoor and Sharma (2016), even if the functions are similar. We believe that this difference could stem from how the spherical harmonics are incorporated for fluctuating vesicles in the two studies. Ahmadpoor and Sharma (2016) do so by using a variational perturbation approximation and a mean field approach to incorporate higher order terms in the potential energy and the resulting statistical averages. We, on the other hand, have to incorporate spherical harmonics through the membrane force in the dynamic equation for the membrane and retain only linear order terms in the expansion of the mean curvature H in terms of u and \underline{u} , which may introduce some artifacts. A more rigorous approach would entail retaining higher order terms and using a mean field approximation to include their effect through statistical averages. This will make an interesting future extension of our results.

6. Conclusion

We have presented a theory for active membranes based on principles of continuum mechanics and investigated the statistical mechanics of active quasi-spherical vesicles. We envision that the general theory developed here will provide a unifying continuum mechanics framework to understand and build upon seemingly disparate but insightful prior studies on active membranes. We modeled the non-equilibrium behavior of active membranes by investigating their relaxation dynamics in a dissipative medium and examined their steady state behavior. We utilized active noise terms to model the impact of active proteins but the theory is general and can be used with other proposed forms for active forces. We have also presented a discussion on how surface tension is incorporated in the models for the dynamics of membranes which we hope will provide a context for the different treatments of surface tension found in the literature.

A specific contribution of our work is that we have extended the continuum model by considering nonlinear curvature elasticity, thus going beyond the existing studies on active membranes based on the classical Helfrich–Canham energy functional. A key analytical result of our study is that nonlinear curvature elasticity impacts the statistical mechanics of active membranes through the bending energy and the surface tension. Comparing tense and tensionless membranes, we reveal that surface tension plays a more dominant role in reducing the fluctuations in comparison with active forces and constitutive nonlinearity. Although many theoretical studies on passive membranes ignore surface tension as a parameter that simply accounts for the areal constraint, our findings indicate that it would be worthwhile to consider surface tension and its interplay with activity for a judicious treatment of active membranes. A highlight of our study is that we use the fluctuation spectrum of active vesicles to understand their vesicle size distribution based on a simplifying assumption that the active vesicles are in thermal equilibrium. An interesting implication of the numerical results is that active vesicles can attain different size distributions which are not possible for passive vesicles.

The continuum mechanics based approach developed here also opens avenues to study the role of active membranes in a host of biophysics problems such as entropic interactions, interactions with inclusions, role of cytoskeleton, and pore formation. The framework also provides a systematic way for enriching the theoretical model with additional physics such as electromechanical coupling. Since we were able to include nonlinear curvature elasticity in our theory by retaining only linear terms in the expansion of the potential energy in spherical harmonics, a more rigorous study could consider higher order terms through the use of a mean field approximation. Although the stability of passive membranes has been a subject of interest in the past, understanding the role of active forces in governing the stability of active membranes presents a fascinating future study.

Finally, a fundamental question in the context of active matter is how far are they from thermodynamic equilibrium. Our work, and all prior studies for that matter, focus on the steady state behavior of active membranes based on the central assumption that the active membranes are close to equilibrium. But are they? This is an open question which requires advanced experimental and theoretical studies rooted in non-equilibrium statistical mechanics.

CRediT authorship contribution statement

Yashashree Kulkarni: Conceptualization, Funding acquisition, Formal Analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing.

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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