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Citation: *The Journal of Chemical Physics* **141**, 024901 (2014); doi: 10.1063/1.4885340

View online: <http://dx.doi.org/10.1063/1.4885340>

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Peptide-induced membrane curvature in edge-stabilized open bilayers: A theoretical and molecular dynamics study

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(Received 16 April 2014; accepted 11 June 2014; published online 8 July 2014)

Peptide- or protein-induced curvatures of lipid membranes may be studied in molecular dynamics (MD) simulations. In these, membranes are usually modeled as infinitely extended bilayers by using periodic boundary conditions. However, the enforced periodicity results in an underestimation of the bending power of peptides, unless the patch size is much larger than the induced curvature radii. In this letter, we propose a novel approach to evaluate the bending power of a given distribution and/or density of peptides based on the use of flat open-edged lipid patches. To ensure long-lived metastable structures, the patch rim is stabilized in MD simulations by a local enrichment with short-chain lipids. By combining the theory of continuum elastic media with MD simulations, we prove that open-edged patches evolve from a planar state to a closed vesicle, with a transition rate that strongly depends on the concentration of lipid soluble peptides. For close-to-critical values for the patch size and edge energy, the response to even small changes in peptide concentration adopts a transition-like behavior (buckling instability). The usage of open-edged membrane patches amplifies the bending power of peptides, thereby enabling the analysis of the structural properties of membrane-peptide systems. We applied the presented method to investigate the curvature induced by aggregating β -amyloid peptides, unraveling a strong sensitivity of membrane deformation to the peptide concentration.

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I. INTRODUCTION

A. Motivation

Foreign substances (e.g., drugs, peptides, or proteins) may induce a concentration-dependent curvature of planar lipid bilayers.^{1–3} An appropriate way to investigate the induced membrane strain is to perform molecular dynamics (MD) simulations.^{2,4,5} However, observed membrane deformations may be sensitive to the size of the studied membrane patch, and particularly also depend on the usage of periodic boundary conditions (PBCs). PBCs are frequently used together with membranes extending from edge to edge, i.e., PBCs allow for the study of infinitely extended membranes, albeit on the cost of periodicity and undulations that are restricted to small wavelengths and wave heights. This procedure grossly underestimates curvature effects, unless the box size is prohibitively large for MD simulations with box lengths much larger than the involved curvature radii.

Here, in order to characterize the mechanical stress induced on the membrane both due to single membrane proteins and in particular during the first stages of protein aggregation we developed a non-conventional procedure making use of simulations of finite membrane systems to obtain a more realistic membrane response without appreciably changing the physical nature of the system. A combined novel analytical and computational approach exploits the well-known

phenomenon of buckling instability arising in loaded elastic bodies. Theoretically, buckling is caused by a bifurcation in the solution to the equations of static equilibrium. At a certain stage under an increasing applied load, further load is able to be sustained in one of two equilibrium states: an undeformed state or a laterally deformed state. We developed a simple theory for the self-buckling of large lipid-protein membrane patches with its boundary exposed to water. The unfavorable energy of the rim and the associated line tension (playing the role of a heavy external load) pushes the evolution of the system from a planar shape to a closed vesicle, against the costly membrane bending energy. In addition to the analytical approach, we suggest the study of finite systems in coarse-grained (CG) MD simulations. Different to previous all-atom approaches of finite systems on a timescale of tens of nanoseconds,^{6–8} we apply coarse-grained simulations with a tunable rim energy (see below) to gain useful insight into the action of, e.g., peptides on bilayer properties on the microsecond timescale.

Our approach exploits two advantageous properties that characterize the buckling instabilities:

First, the phenomenon of buckling instabilities highlights a huge transition-like response of the membrane curvature against any perturbation in the elastic structure. The engineering and scientific literature contains a lot of papers dealing with the sudden and catastrophic failure of elastic structures under the effect of applied loads. Many geometrical arrangements, elastic parameters, and various kinds of applied loads have been explored so far (among the many

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FIG. 1. Molecular dynamics simulations of evolving planar bilayers (grey) doped with lipid soluble proteins (yellow).

review works on this subject see, e.g., Ref. 9). Extension of these concepts to bio-medical applications¹⁰ and to micro- and nano-scale systems (e.g., Langmuir-Blodgett molecular films,^{11,12} molecular graphene sheets,¹³ viral capsids,¹⁴ supercoiled DNAs,¹⁵ lipid membranes^{16,17}) have also been developed. While the onset of a buckling instability (and the following post-buckling behavior) is often considered a harmful event, engineers have learned how to turn drawbacks into opportunities. The amplified response to loads has been employed in developing pressure measurement devices. For instance, the controlled buckling of multiwalled carbon nanotubes has been used as a tool for pressure, tactile, and vibration sensing.¹⁸ A different, but conceptually related method, has been applied to measure the elastic modulus of polymers via a buckling-related metrology.^{19,20}

Second, it is well-known that buckling instabilities are extremely sensitive to local geometrical or elastic defects or to load misalignment. Such a behavior has been observed both in macroscopic (see, e.g., Ref. 21) and mesoscopic²² systems. Again, this peculiar behavior poses big troubles to engineers since the load-carrying capacity of structures is significantly affected by the presence of physical imperfections that may lead to catastrophic failures, even in the case of small loads. On the other hand, we believe that the extreme sensitivity of the buckling instabilities to local imperfections might turn useful in investigating tiny variations of the membrane curvature upon protein concentration and aggregation.

B. Doped flat bilayers with free boundaries

In Sec. I A, we have shown how buckling instabilities may unravel hidden static and dynamic properties of mesoscopic and macroscopic bodies. Here, these concepts are applied to the interesting field of biological membranes.

As said in Sec. I A, MD simulations of impurity-induced membrane deformations are sensitive to the size of the simulation box when conventional periodic boundary conditions with infinitely extended membranes are used, i.e., no membrane edges are present. This procedure may largely underestimate curvature effects, depending on the size of the periodic system as it will be shown in Sec. II C by a classical theory of continuum elastic media and in Sec. III D by using MD simulations of both finite and infinite membrane systems. The simplicity of the analytical model ensures an easy understanding of the relationship between membrane deformation and the bending power of the impurities.

In order to obtain a more realistic picture of the mechanical membrane deformation during protein insertion and/or ag-

gregation from simulations, we developed a non-conventional procedure that does not appreciably change the physical nature of the system. Specifically, we consider a finite lipid bilayer with its outmost boundary exposed to water. The unfavorable energy of the rim renders this system metastable. The unfavorable line tension (a tunable parameter estimated from both theory and experiments) favors the evolution of a planar shape bilayer towards a closed vesicle as shown in Fig. 1. Borrowing an effect commonly observed in nature,²³ the tuning of the edge energy in MD simulations has been achieved by adding short-chain phospholipids to the strongly curved bilayer edge. While the evolution towards a closed vesicle reduces the length of the water-exposed rim, the membrane bending upon vesiculation is energetically costly. The balance between line tension and bending elasticity gives rise to a maximum in the potential energy when plotted against the bending angle Φ . A representative curve is shown in Fig. 2.

Two points are worth mentioning: (i) The thermodynamically stable state is the closed vesicle, and (ii) the transition planar bilayer $\rightarrow$ vesicle is an energy activated process, i.e., bending fluctuations with an amplitude $\Phi \geq \Phi_0$ irreversibly evolve towards a vesicle structure, while the planar state is retained for smaller deformations.

By employing an oversimplified theoretical model we shall see in Sec. II A how the energy barrier h for the transition from a metastable planar state to the vesicle state depends on the line tension, the bending rigidity (constant

FIG. 2. Sketch of the energy curve for the deformation of a finite membrane from a planar bilayer with free boundaries exposed to water to a closed vesicle. The total free energy – considering the rim energy and the bending energy – is plotted against the bending deformation Φ from planarity.

parameters in most cases) and, more importantly, on the *bending power* of the impurities.

Because of the barrier h , the transition from a metastable planar bilayer to a closed vesicle is a rare event. The first passage time $\tau(\Phi_0)$ – i.e., the time required for a stochastic process to reach a preassigned value Φ_0 – can easily be calculated by standard non-equilibrium methods. It is exponentially related to the energy barrier h and the temperature. In turn, as shown in Sec. II A, the barrier h is proportional to the *bending power* of the impurities. Some numerical estimates will be provided in Sec. II B.

Unfortunately, this exactly solvable model is plagued by the assumption of a constant spontaneous curvature over the whole bilayer surface. Such an approximation prevents one to study subtle effects such as the role of aggregating membrane proteins inducing membrane deformation. In Sec. II D, a more refined model based on the linear response theory of elastic media will be developed. This model has the considerable advantage of being sensitive to the spatial distribution of impurities.

In Sec. III, we will compare our theoretical results with extensive MD simulations performed using the CG Martini force field.²⁴ Finally, results, open questions, and future developments of the applied methods will be discussed in Sec. IV.

II. THEORY

A. Modeling the planar to curved bilayer transition

Consider a circular lipid patch with its edge exposed to water. In the following, a simple model to calculate the free energy of this metastable arrangement and its evolution towards a stable spherical vesicle is devised. The general solution of this formidable problem is very difficult and several papers dealing with this issue have been written over the time.^{25–29} Here, we follow a simple approximate procedure that retains most of the relevant physics. A similar procedure, that does not include the relevant spontaneous curvature effects, has been developed some time ago. The total energy of the system, G_{tot} , can be partitioned into the sum of three contributions: an elastic, a surface, and an edge contribution

$$G_{tot} = G_{elastic} + G_{surf} + G_{edge}. \quad (1)$$

The elastic term can be described in the framework of the Helfrich picture³⁰ of membrane elastic deformations

$$G_{elastic} = \int_S \left[\frac{1}{2} \kappa_b (c_1 + c_2 - c_0)^2 + \kappa_G c_1 c_2 \right] dS, \quad (2)$$

where κ_b and κ_G are the bending modulus and the Gaussian curvature modulus, respectively, c_1 and c_2 are the two local principal curvatures and c_0 is the spontaneous curvature. The integration spans over the whole surface S of the membrane patch. Assuming a constant and uniform deformation of the patch, we may set $c_1 = c_2 = 1/R$ (and $c_0 = 1/R_0$) with $1/R$ as the curvature of the lipid patch. In symmetric lipid bilayers $1/R_0 = 0$, the asymmetric inclusion of foreign molecules between the two monolayers induces a spontaneous curvature

FIG. 3. Schematic drawing of a closing bilayer with water-exposed edge.

c_0 . It strongly depends on the differential area increase between the two leaflets upon insertion and has been related to shape and concentration of inclusions by several authors (see, e.g., Refs. 31–33). Moreover, we assume that the curved patch retains its own surface area during bending deformation, i.e., $\int_S dS = \frac{1}{2} NA$ (N being the number of lipids and A the constant surface area per lipid). With the aid of the above formulas we write down the elastic energy

$$G_{elastic} \approx \left[\frac{1}{2} \kappa_b \left(2 - \frac{R}{R_0} \right)^2 + \kappa_G \right] \frac{NA}{2R^2}. \quad (3)$$

The surface energy G_{surf} is given by

$$G_{surf} = \int_S \gamma_S dS \quad (4)$$

with γ_S as the membrane-water surface tension. The incompressibility condition enforces that this term remains constant. Therefore, it can be safely adsorbed into an unessential additive numerical constant. Finally, the unfavorable edge energy G_{edge} is related to the line tension γ_l and to the contour length $l = 2\pi a$ exposed to water (see Fig. 3)

$$G_{edge} = \oint_l \gamma_l dl = 2\pi \gamma_l a. \quad (5)$$

The parameter γ_l can be inferred from experimental data (10–50 pN, see, e.g., Ref. 34) or calculated from MD simulations (see, e.g., Refs. 35–39). Simple geometry relates a with the curvature radius R and the maximum perpendicular deformation h of the bent patch, $h = R - \sqrt{R^2 - a^2}$

$$G_{edge} = 2\pi \gamma_l [R^2 - (R - h)^2]^{1/2}. \quad (6)$$

Combining Eqs. (3) and (6), using the relationship $\int_S dS = 2\pi Rh = \frac{1}{2} NA$, and introducing the radius D of the patch in

its reference planar arrangement, $D = (\frac{1}{2\pi}NA)^{1/2} = \text{const.}$, and $x = h/D$, we obtain

$$G_{tot} = \text{const.} + 4\pi \left\{ (2\kappa_b + \kappa_G)x^2 + \frac{\kappa_b}{8} \frac{D^2}{R_0^2} - \kappa_b \frac{D}{R_0}x + \frac{1}{2}\gamma_l D[1-x^2]^{1/2} \right\}. \quad (7)$$

Differentiation with respect to x reveals a maximum of the total energy in Eq. (7). It is determined by solving the algebraic equation

$$A_1 - (1-x^2)^{-1/2} = A_2 x^{-1} \quad (8)$$

with

$$A_1 = \frac{4(2\kappa_b + \kappa_G)}{\gamma_l D} \quad (9)$$

and

$$A_2 = \frac{2\kappa_b}{\gamma_l R_0}. \quad (10)$$

Albeit Eq. (8) can be easily solved by numerical techniques, it is preferable to employ a perturbation procedure in the small parameter A_2 (small intrinsic curvature of the lipid patch). This procedure will lead to simple and transparent results as shown below. Solving Eq. (8) for x yields for the leading terms

$$x \approx \left(1 - \frac{1}{A_1^2}\right)^{1/2} - \frac{A_2}{A_1(A_1^2 - 1)} + \dots \quad (11)$$

Neglecting the second term in the above equation, shows that real solutions for x exist for $A_1 > 1$, i.e.,

$$A_1 = \frac{4(2\kappa_b + \kappa_G)}{\gamma_l D} > 1. \quad (12)$$

The above inequality is satisfied for large bending rigidity (both κ_b and κ_G), small patch sizes D , and small line tension γ_l . Inserting typical values for DPPC bilayers (compare Sec. II B) yields a critical patch size of $D \approx 15$ – 30 nm. When the inequality is not satisfied, a sudden barrierless transition from a planar state to a concave arrangement takes place. If patches are large enough, they will further evolve towards a close spherical vesicle where no free edges are exposed to water.

Inserting the above result into the general expression for the total energy, Eq. (7), yields the energy of the transition barrier

$$G_{tot}^* = \text{const.} + 2\pi\gamma_l D \left[\frac{A_1^2 + 1}{2A_1} + A_3 - \frac{A_2}{A_1}(A_1^2 - 1)^{1/2} \right] \quad (13)$$

with

$$A_3 = \frac{\kappa_b D}{4\gamma_l R_0^2}. \quad (14)$$

Once the barrier height has been calculated, the first passage time starting from the initial planar state (i.e., the total energy at $x = 0$) is easily evaluated to

$$k \approx \nu \exp\left(-\frac{\Delta G}{k_B T}\right) \quad (15)$$

with the pre-exponential factor or attempt frequency ν and $\Delta G = G_{tot}^* - G_{tot}(x = 0)$. It is trivial to see from Eq. (7) that $G_{tot}(x = 0) = \text{const.} + 2\pi\gamma_l D(A_3 + 1)$. Rearranging Eq. (15) into the more useful form

$$\log(k) \approx \log(\nu) - C_1 + C_2/R_0, \quad (16)$$

with

$$C_1 = 4\pi \frac{2\kappa_b + \kappa_G}{k_B T} \cdot \frac{(A_1 - 1)^2}{A_1^2}, \quad (17)$$

and

$$C_2 = 4\pi \frac{\kappa_b}{k_B T} D \cdot \frac{(A_1 - 1)^{1/2}}{A_1}. \quad (18)$$

This relationship links the transition rate k to the spontaneous curvature R_0 . In turn, R_0 is related to the concentration c of curvature-inducing foreign molecules in the lipid bilayer: $R_0 \equiv R_0(c)$. In case of vanishing concentration c , the bilayer curvature goes to infinity, $R_0(c = 0) \rightarrow \infty$, and the rate reads $\log(k(c = 0)) = \log(\nu(c = 0)) - C_1$. Combining the above expressions we obtain

$$\log \frac{k(c)}{k(c = 0)} \approx \frac{C_2}{R_0(c)}, \quad (19)$$

where the small logarithm corrections coming out from the pre-exponential term have been neglected.

Equation (19), together with Eq. (12), are the main results of the above theoretical framework. It shows that the logarithm of the rate for the transition from a planar lipid patch to a curved arrangement (and eventually to a closed vesicle) is, under favorable conditions dictated by Eq. (12), a barrier-controlled process. Moreover, the transition rate sharply increases with the strength of the bilayer spontaneous curvature $1/R_0$. The sensitivity of the bilayer response to spontaneous curvature effects is measured by the parameter C_2 in Eq. (19) that depends on the bending modulus, the line tension, and the size of the lipid patch (all of them, in principle, can be determined experimentally, see Sec. II B).

B. Numerical calculations and results of the simplified model

Here, we briefly list some numerical estimates of the parameters entering the above equations. The bending modulus of a lipid bilayer is typically of the order of $20 k_B T$, a value strongly depending on the lipid chain length, the unsaturation degree, and the surface charge.^{40–49} The Gaussian curvature modulus κ_G does not differ too much from κ_b : $\kappa_G \approx 0.7$ – $0.9 \cdot \kappa_b$.⁵⁰ The line tension γ_l can be inferred from experimental data ($\gamma_l \approx 10$ – 50 pN, see, e.g., Ref. 34) or calculated by analytical techniques⁵¹ and from MD simulations.^{35–39}

Using the above values we calculated from Eq. (12) the critical radius D_{crit} of a lipid bilayer patch: for $D < D_{crit}$ one may trap the bilayer into a metastable flat configuration for a time sufficiently long for observation, while the system is unstable for $D > D_{crit}$ and immediately evolves towards a curved arrangement. A rough estimate yields $D_{crit} \approx 15$ – 30 nm for DPPC bilayers. In practice, smaller sizes have to be considered: for sizes D near D_{crit} the energy barrier is low,

therefore the residence time into the metastable state may be too short to be detected. Spontaneous curvature effects even decrease the critical radius due to their lowering of the energy barrier.

As said in the Introduction, small patch sizes (10^{-8} – 10^{-9} m) are not representative for most biological systems. Suppose we want to study by MD simulations the bending power of a distribution of membrane proteins and how the membrane deformation changes with the protein concentration. The solution of this problem requires larger lipid patches that remain trapped into a planar long-living metastable state. One way out could be to artificially increase the membrane bending rigidity. This approach will alter the membrane properties as well as the lipid-membrane interactions. A different possibility used in this study is to lower the line tension at the bilayer edge by adding a small amount of short chain lipids that preferentially bind at the far edge of the lipid bilayer. This approach mimics the well-known behavior of short-chained lipids and soaps that stabilizes with great efficiency the water-exposed rims of lipid membranes.^{52,53} The resulting structures (often referred to as *bicelles*) are stable and can be used as membrane model systems.²³ Conceptually, the stabilizing short-chain lipids are similar to linactant lipids that reduce the line tension at the interface between different lipid domains, e.g., between liquid-ordered and liquid-disordered domains in biological membranes.⁵⁴

This approach appears more suitable to study model systems because the perturbation is localized in a narrow region at the patch periphery and does not significantly perturb the strength of lipid-lipid and lipid-protein interactions.

Once a long-living metastable patch has been built, Eq. (19) allows to estimate the concentration-dependent curvature power of a curvature-inducing foreign molecule. This task can be accomplished by measuring in MD simulations the transition rate kinetics (or, more precisely, the first passage time) at increasing concentrations c of curvature-inducing molecules. The ratio between the logarithm of the transition rates $\log \frac{k(c)}{k(c=0)}$ and the parameter C_2 (see above) yields the concentration-dependent curvature $1/R_0(c)$.

Let us briefly discuss the main limitations of the above simplified model. Its major drawback is the assumption of a unique curvature radius to describe membrane deformations. Especially in inhomogeneous bilayers the local curvature strongly depends on the composition. So, at the best, we may consider the model as a mean field picture, where local curvatures are smeared out and averaged into a unique non-local parameter. In Sec. II C, we will remove this severe assumption and explicitly calculate the local membrane deformations.

C. Bending deformation of an infinite planar bilayer by curvature-inducing molecules under periodic boundary conditions

Consider a slightly distorted planar membrane patch. Let η be a small local deformation from planarity, the total energy can be described as the sum of the membrane elastic deformation energy plus a spontaneous curvature term, $C_0(x, y)$, originating from the curvature-induced foreign

molecules freely diffusing in the lipid bilayer. These two terms, lumped together into Eq. (2), can be expressed as a function of η ³⁰

$$G_{\text{elast}} = \int_S \left[\frac{1}{2} \kappa_b \left(\frac{\partial^2 \eta}{\partial x^2} + \frac{\partial^2 \eta}{\partial y^2} - C_0(x, y) \right)^2 + \kappa_G \left(\frac{\partial^2 \eta}{\partial x^2} \frac{\partial^2 \eta}{\partial y^2} - \left(\frac{\partial^2 \eta}{\partial x \partial y} \right)^2 \right) \right] dx dy, \quad (20)$$

where only quadratic terms have been retained. The local curvature term $C_0(x, y)$ has been assumed to be proportional to the local concentration $c(x, y)$ of foreign molecules in the bilayer

$$C_0(x, y) \approx \left. \frac{\partial C_0}{\partial c} \right|_{c=\bar{c}} \cdot c(x, y). \quad (21)$$

The local concentration must satisfy the mass conservation condition: $\int_S c(x, y) dx dy = n$, with n the total number of curvature-inducing molecules. If the distribution of foreign molecules is axisymmetric, i.e., $c(x, y) = c(r)$ with $0 < r < D$, then a suitable distribution with a maximum in the center of the patch reads: $c(r) = A - \lambda r^2 = A - \lambda(x^2 + y^2)$. By exploiting the mass conservation condition, we eliminate the unknown A obtaining

$$C_0 \approx \left. \frac{\partial C_0}{\partial c} \right|_{c=\bar{c}} \cdot \left[\bar{c} - \lambda \left(x^2 + y^2 - \frac{1}{2} D^2 \right) \right], \quad (22)$$

where $\bar{c} \equiv n/\pi D^2$ is the mean density of foreign molecules in the bilayer. The constant factor $\left. \frac{\partial C_0}{\partial c} \right|_{c=\bar{c}}$ (which has the dimension of a length) contains all the details of the bending power of a single isolated foreign molecule, it can be determined by experiments or by molecular modeling of the impurity-bilayer system. Typical values of $\bar{c}$ are 1 molecule/nm², hence, for a membrane patch radius of about 10^{-8} – 10^{-7} m, λ cannot exceed 10^{32} m⁻⁴.

Minimization of the total free energy is performed following the standard variational procedure that leads to the Euler-Lagrange equation⁵⁵

$$\frac{\partial G_{\text{tot}}}{\partial \eta} - \frac{\partial}{\partial x} \frac{\partial G_{\text{tot}}}{\partial \eta_x} - \frac{\partial}{\partial y} \frac{\partial G_{\text{tot}}}{\partial \eta_y} + \frac{\partial^2}{\partial x^2} \frac{\partial G_{\text{tot}}}{\partial \eta_{xx}} + 2 \frac{\partial^2}{\partial x \partial y} \frac{\partial G_{\text{tot}}}{\partial \eta_{xy}} + \frac{\partial^2}{\partial y^2} \frac{\partial G_{\text{tot}}}{\partial \eta_{yy}} = 0, \quad (23)$$

where $\eta_x \equiv \partial \eta / \partial x$ and $\eta_{xx} \equiv \partial^2 \eta / \partial x^2$. After simple algebra we obtain

$$\nabla^2 \nabla^2 \eta = \nabla^2 C_0, \quad (24)$$

where $\nabla^2 \equiv \partial^2 / \partial x^2 + \partial^2 / \partial y^2$. It is worth noting that η does not depend any longer on the elastic modulus of the Gaussian curvature κ_G .

Adopting cylindrical polar coordinates and exploiting Eq. (22), we derive from Eq. (23)

$$\nabla^2 \left[\nabla^2 \eta - \bar{C}_0 + B \left(r^2 - \frac{1}{2} D^2 \right) \right] = 0 \quad (25)$$

with

$$\nabla^2 \equiv \frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial}{\partial r} \right), \quad (26)$$

$$\overline{C}_0 \equiv \left. \frac{\partial C_0}{\partial c} \right|_{c=\bar{c}} \cdot \bar{c}, \quad (27)$$

and

$$B \equiv \left. \frac{\partial C_0}{\partial c} \right|_{c=\bar{c}} \cdot \lambda. \quad (28)$$

Integrations of Eq. (25) lead to

$$\eta = Q_1 + Q_2 r^2 + Q_3 \log \frac{r}{D} + Q_4 r^2 \log \frac{r}{D} + \frac{1}{4} \overline{C}_0 r^2 - \frac{1}{16} B r^2 (r^2 - 2D^2). \quad (29)$$

Q_1 , Q_2 , Q_3 , and Q_4 are four integration constants to be determined. We imposed that the membrane deformation profile satisfies PBCs at the edges of a large imaginary box containing the fluctuating membrane. This choice has been done in order to compare our theoretical with MD data. Boundary conditions are:

1. Symmetry imposes that at the center $r = 0$ the first derivative vanishes: $\partial\eta/\partial r|_{r=0} = 0$.
2. At $r = 0$, the second derivative $\nabla^2\eta$ must always assume a finite value.
3. By symmetry, at $r = 0$ the membrane deformation reaches its largest value. Without loss of generality, the membrane deformation is measured with respect to its maximum value occurring at $r = 0$, namely, $\eta|_{r=0} = 0$.
4. By symmetry, PBCs at the edge of the simulation box impose that: $\partial\eta/\partial r|_{r=D} = 0$.

The boundary conditions 1–3 lead to

$$Q_1 = Q_3 = Q_4 = 0. \quad (30)$$

In order to satisfy the condition at the far edge of the membrane patch, $\partial\eta/\partial r|_{r=D} = 0$, we obtain from Eq. (29)

$$Q_2 = -\frac{1}{4} \overline{C}_0. \quad (31)$$

Combining the above results, eventually one arrives at a very simple result

$$\eta(r) = -\frac{1}{16} \left. \frac{\partial C_0}{\partial c} \right|_{c=\bar{c}} \cdot \lambda r^2 (r^2 - 2D^2). \quad (32)$$

The first term, $\partial C_0/\partial c|_{c=\bar{c}}$, describes the variation of the membrane curvature triggered by a constant concentration $\bar{c}$ of curvature-inducing molecules randomly distributed over the membrane plane. For any class of molecules, $\partial C_0/\partial c|_{c=\bar{c}}$ can be deduced from experiments or obtained from MD simulations. In any case, models for induced curvature show that $\overline{C}_0$ is inversely related to the membrane rigidity. The term λ accounts for the effect of molecules distribution on the membrane curvature: when $\lambda = 0$ the distribution of curvature-inducing molecules is homogeneous, large λ values instead

FIG. 4. Membrane deformation profile (blue lines) and concentration profile (red lines) of curvature-inducing molecules within the membrane. Data are expressed in arbitrary units.

imply a large concentration at the center of the lipid patch. A typical membrane deformation profile is reported in Fig. 4 (blue lines) where we report the deformation from planarity around a patch containing foreign molecules. We assumed a axisymmetric distribution, so the profile depends only on the radial distance r measured from the center of the patch. In the same figure, we also report the concentration profile of the curvature-inducing molecules (red lines). Curves (expressed in arbitrary units) have been calculated through Eq. (32) by maintaining constant the number n of foreign molecules but considering increasing clustering of the molecules. As expected, when the molecules form denser clusters the curvature effect is more pronounced.

D. Bending deformation of a free-edge bilayer by curvature-inducing molecules

At variance of the model developed in Sec. II C, in the presence of water-exposed edges in lipid bilayers, we have to add the unfavorable line tension energy to the classic elastic contribution described by Eq. (20). As shown in Sec. II A, for axisymmetric deformed shapes the edge energy reads $G_{edge} = \oint \gamma_l dl = 2\pi\gamma_l a$ (see Fig. 3), where γ_l is the line tension. Generally speaking, the contour length $2\pi a$ is smaller than the contour length of the undeformed planar bilayer. The shortening of a during the bending deformation is the clue that explains why open bilayers gain energy during the vesiculation process. For a spherical cup deformation, a has been calculated in Sec. II A, here we generalize the above equations for a generic axisymmetric deformation of an open bilayer. We start from the conservation of the bilayer's surface area upon any deformation η , i.e.,

$$\int_S \left[1 + \left(\frac{\partial \eta}{\partial x} \right)^2 + \left(\frac{\partial \eta}{\partial y} \right)^2 \right]^{1/2} dx dy = \pi D^2, \quad (33)$$

where πD^2 is the surface area of the planar bilayer. For small deformations from planarity, we expand Eq. (33) in power

series of η

$$\int_S dx dy + \frac{1}{2} \int_S \left[\left(\frac{\partial \eta}{\partial x} \right)^2 + \left(\frac{\partial \eta}{\partial y} \right)^2 \right] dx dy = \pi D^2. \quad (34)$$

The first term $\int_S dx dy = \pi a^2$ describes the projected area of a crumpled membrane onto the x-y plane. Solving the above equation for a and expanding again the result in power series of η , we find up to quadratic terms

$$a \approx D - \frac{1}{4\pi D^2} \int_S \left[\left(\frac{\partial \eta}{\partial x} \right)^2 + \left(\frac{\partial \eta}{\partial y} \right)^2 \right] dx dy. \quad (35)$$

This result shows an overall reduction of the contour length with membrane crumpling. Therefore, the edge energy of a slightly deformed bilayer reads

$$G_{edge} = 2\pi\gamma_l a \approx 2\pi\gamma_l D - \frac{\gamma_l}{2D^2} \int_S \left[\left(\frac{\partial \eta}{\partial x} \right)^2 + \left(\frac{\partial \eta}{\partial y} \right)^2 \right] dx dy. \quad (36)$$

Notice that the last term is always negative: edge tension favors a large deformation from planarity. The total energy is given by adding the edge energy Eq. (36) to the elastic energy Eq. (20)

$$G_{tot} = G_{elastic} + G_{edge}. \quad (37)$$

Minimization of the total free energy Eq. (37) through the Euler-Lagrange equation and usage of cylindrical polar coordinates leads to

$$\nabla^2 \nabla^2 \eta = \nabla^2 (C_0(r) - \mu^2 \eta) \quad (38)$$

with $\mu^2 = \frac{\gamma_l}{\kappa_b D^2}$, while the local spontaneous curvature reads: $C_0(r) \equiv \bar{C}_0 - B(r^2 - \frac{1}{2}D^2)$. As we shall see shortly later, Eq. (38) may present a complex behavior and, in particular, the phenomenon of *buckling instability*, i.e., a sudden transition from a planar to a bent arrangement.⁹ A first integration of Eq. (38) leads to

$$\nabla^2 \eta + \mu^2 \eta = C_1 + C_2 \log \frac{r}{D} + C_0(r), \quad (39)$$

C_1 and C_2 are integration constants. The solution of Eq. (39) is given by

$$\eta = \frac{1}{\mu^2} C_1 + C_2 g_1(r) + g_2(r) + C_3 J_0(\mu r) + C_4 Y_0(\mu r), \quad (40)$$

where $J_0(x)$ and $Y_0(x)$ are Bessel's functions of the first and second kind, respectively, while C_1 , C_2 , C_3 , and C_4 are four integration constants to be determined. The functions $g_1(r)$ and $g_2(r)$ are defined as follows:

$$g_1(r) = \frac{\pi}{2} \int_r [Y_0(\mu r) J_0(\mu r') - J_0(\mu r) Y_0(\mu r')] \log \left(\frac{r'}{D} \right) r' dr', \quad (41)$$

$$g_2(r) = \frac{\bar{C}_0}{\mu^2} - f(r), \quad (42)$$

$$f(r) = \frac{\pi}{2} B \cdot \int_r [Y_0(\mu r) J_0(\mu r') - J_0(\mu r) Y_0(\mu r')] \left(r'^2 - \frac{1}{2} D^2 \right) r' dr'. \quad (43)$$

In deriving the above equations, the relationship $J_\nu(x) Y_{\nu+1}(x) - J_{\nu+1}(x) Y_\nu(x) = -\frac{2}{\pi x}$ has been repeatedly used. The boundary conditions for a free edge bilayer differ from the periodic ones adopted in Sec. II C. Specifically, at the center $r = 0$ of the lipid patch we retained the same three conditions employed above

$$\begin{aligned} \nabla^2 \eta|_{r=0} &= \text{finite}, \\ \eta|_{r=0} &= 0, \\ \frac{\partial \eta}{\partial r} \Big|_{r=0} &= 0, \end{aligned} \quad (44)$$

while at the edge $r = D$ we generalized the standard boundary conditions used for a free-edge plate by adding the effect of the spontaneous curvature $C_0(r)$ ⁵⁶

$$-(\nabla^2 \eta|_{r=D} - C_0(D)) + \frac{\kappa_b - \kappa_G}{\kappa_b} \frac{1}{r} \frac{\partial \eta}{\partial r} \Big|_{r=D} = 0, \quad (45)$$

$$\frac{\partial}{\partial r} (\nabla^2 \eta - C_0(r)) \Big|_{r=D} = 0. \quad (46)$$

In deriving Eq. (45), we compared the Helfrich with the continuum theory of elastic plates to obtain a simple relationship between the Gaussian curvature modulus κ_G , the Poisson ratio σ , and the bending modulus κ_b : $\kappa_G = (1 - \sigma)\kappa_b$. At variance of the periodic boundary conditions case, the identity $\nabla^2 \eta|_{r=0} = \text{finite}$ is always fulfilled over the whole range $0 < r < D$, therefore we have four independent conditions to fix four integration constants.

Boundary conditions at $r = 0$ are calculated by exploiting the properties of the Bessel functions: $J_0(x) \rightarrow 1 - \frac{1}{4}x^2 + \dots$, $Y_0(x) \rightarrow \frac{2}{\pi}(\log(x/2) + \gamma) + \dots$ (with the Euler constant $\gamma = 0.57721$), and $\partial J_0(x)/\partial x = -J_1(x)$, $\partial Y_0(x)/\partial x = -Y_1(x)$. It can also be easily seen that $g_1(0) = f(0) = 0$ and $\partial g_1(r)/\partial r|_{r=0} = \partial f(r)/\partial r|_{r=0} = 0$. In order to avoid the divergence of η and that of its first derivative at $r = 0$, we must set

$$C_4 = 0 \quad (47)$$

while from the boundary condition $\eta|_{r=0} = 0$ we obtain

$$0 = \frac{1}{\mu^2} (C_1 + \bar{C}_0) + C_3. \quad (48)$$

Analogously, by employing the boundary conditions Eqs. (45) and (46) together with Eq. (39) we arrive at

$$\begin{aligned} C_3 \left(J_0(\mu D) - \sigma \frac{J_1(\mu D)}{\mu D} \right) \\ + C_2 \left(g_1(D) + \frac{\sigma}{\mu^2 D} \frac{\partial g_1(r)}{\partial r} \Big|_{r=D} \right) \\ = -\frac{\bar{C}_0}{\mu^2} + f(D) + \frac{\sigma}{\mu^2 D} \frac{\partial f(r)}{\partial r} \Big|_{r=D}, \end{aligned} \quad (49)$$

FIG. 5. Membrane deformation $\Phi \equiv |\eta(D) - \eta(0)|$ against the line tension γ_l calculated for increasing values of the concentration of the curvature-inducing molecules (blue: $\bar{c} = 1.0$, red: $\bar{c} = 2.0$, green: $\bar{c} = 3.0$). Other parameters are: $\bar{c} = 10^{18} \text{ m}^{-2}$, $\lambda = 10^{32} \text{ m}^{-4}$, $\kappa_b = 10^{-18} \text{ J}$, $\sigma = 0.3$, and $D = 1.5 \times 10^{-8} \text{ m}$. The unknown parameter $\partial C_0/\partial c|_{c=\bar{c}}$ enters as a multiplicative coefficient, it merely scales the y-axis but does not change the topology of the curves (we set in all calculations $\partial C_0/\partial c|_{c=\bar{c}} = 10^{-10} \text{ m}$). The vertical line is the same for all the curves.

$$C_3 \mu J_1(\mu D) + C_2 \left(\frac{1}{\mu^2 D} - \frac{\partial g_1(r)}{\partial r} \Big|_{r=D} \right) = - \frac{\partial f(r)}{\partial r} \Big|_{r=D}, \quad (50)$$

where $\sigma = (\kappa_b - \kappa_G)/\kappa_b$. Equations (47)–(50) enable us to calculate the four integration constants C_i , ($i = 1, \dots, 4$).

Once the integration constants have been obtained, the maximum deformation $\Phi \equiv |\eta(D) - \eta(0)|$ calculated with respect to the center $r = 0$ of the membrane patch is easily evaluated. As expected, we found a discrete spectrum of resonances where the fluctuation amplitude diverges for a critical combination of the control parameters. In the following, we restrict our analysis to deformations of the lowest energy.

Specifically, in Fig. 5 we report the smallest deformation Φ against the edge tension γ_l calculated for an increasing value of the concentration of curvature-inducing molecules. All the curves show a typical transition-like behavior on approaching a critical threshold (defined by the vertical bar common to all curves). As expected, the divergence (vesicle closure) is more rapid on increasing the concentration of foreign molecules.

It is worth noting that the divergence of the amplitudes occurs at values of the edge tension five to six orders of magnitude smaller than those measured for lipid bilayers.³⁴ This means that open bilayers cannot exist in the aqueous environment, unless the unfavorable edge tension has been eliminated by addition of specific molecules along the rim.^{52,53}

Calculations not reported here also show that the heterogeneity parameter λ (a measure of the inhomogeneous distribution of curvature-inducing molecules, $\lambda = 0$ means a uniform random distribution) does not play a significant role. Finally, the variation of the membrane deformation against the bending modulus κ_b shows just the opposite behavior of that reported in Fig. 6, namely, the planar state is favored at high membrane rigidities while the bent configuration is more likely in soft systems. Summarizing, bicelles are stabilized by small sizes, a high bending rigidity, a low line tension, and a modest protein-induced spontaneous curvature.

FIG. 6. Membrane deformation $\Phi \equiv |\eta(D) - \eta(0)|$ against the line tension γ_l calculated for increasing values of the membrane patch radius (green: $D = 10 \text{ nm}$, blue: $D = 20 \text{ nm}$, red: $D = 30 \text{ nm}$). The concentration of curvature-inducing molecules was $\bar{c} = 10^{-18} \text{ m}^{-2}$, other parameters are as in Fig. 5.

III. MOLECULAR DYNAMICS SIMULATIONS

Protein aggregation within the membrane could mark the first step in the multifaceted problem of the action of β -amyloid protein^{57–59} in the Alzheimer disease. Aggregation has been evidenced to be associated with a huge deformation of the membrane,⁶⁰ a phenomenon already observed in several lipid-protein systems.⁶¹

Here, in order to amplify the curvature stress induced on the membrane during the first stages of protein aggregation, we performed CG MD simulations of helical A β -peptides at varying concentration, randomly embedded in a finite phospholipid bilayer stabilized at the rim by short lipids.

A. The Martini model

The CG Martini force field²⁴ uses a mapping of on average four heavy atoms to one CG interaction site. The forcefield has been primarily parameterized to match thermodynamic quantities such as the free energy of hydration, solvation, and partitioning between polar and apolar solvents. The non-bonded interactions were modeled by a shifted 12-6 Lennard-Jones (LJ) potential for the van der Waals interactions and by a shifted Coulomb potential for electrostatic interactions with an implicit dielectric ($\epsilon = 15$) to mimic the screening of water. In this work, we used the literature parameters for water and ions, phosphatidylcholine (POPC), protein, and cholesterol (CHOL).⁶²

Shortened DHPC lipids, made up of 6 beads (Q0, Qa, Na, Na, C1, C1) were used to stabilize the rim of the patch. Additionally, the non-bonded LJ interactions between the short lipid tails with POPC tails and CHOL have been modified to increase *ad hoc* the stability of the open bilayer, by avoiding possible mixing between short lipids at the rim and the investigated membrane patch (destabilizing effect). The strength of the interaction, determined by the value of the LJ well-depth ϵ_{ij} , depends on the interacting particle types i and j . The stability of the patch was increased when a decreased LJ interaction was used between the lipid tails of POPC/CHOL and the short rim lipids with a value of $\epsilon_{ij} = 2.7 \text{ kJ mol}^{-1}$. In

contrast, the standard LJ potential with $\epsilon_{ij} = 3.5 \text{ kJ mol}^{-1}$ had a destabilizing effect on the membrane (see Figure 8). For matter of simplicity, we will refer later on to the former rim lipid as *stabilizing* and the latter lipid as *destabilizing*. Other parameters and topology files were obtained from the Martini website (<http://cgmartini.nl>).

The structure of the A β 42 peptide in an apolar environment (similar to the membrane environment) was obtained from the Protein Data Bank (entry 1IYT⁶³). The mostly helical structure was converted to a coarse-grained representation.

B. Simulation parameters

All simulations were run using version 4.6.1 of Gromacs.⁶⁴ Systems a–d (finite membrane systems, see Sec. III C for a full description) were equilibrated in the presence of short rim lipids in the solvent for 1 μs while peptides, POPC lipids, and cholesterol were kept under constraints. After adhesion at the free rim, the short lipids in excess were removed. This shielding of the bilayer rims stabilizes the finite membrane system.^{34,51,65} All the systems were then equilibrated in a NVT ensemble for 100 ns by using the Berendsen weak coupling thermostat⁶⁶ with a coupling constant of 1.0 ps. After equilibration, the temperature was kept constant at 300 K using the v-rescale algorithm⁶⁷ with a time constant of 1.0 ps. The Parrinello-Rahman algorithm⁶⁸ was applied for semi-isotropic pressure coupling (1 bar) for systems e–h (infinite membrane systems), while we chose to simulate the systems a–d in a NVT ensemble to avoid shrinking of the box that could afterwards potentially stabilize the patch by establishing non-bonded contacts across the periodic boundaries. The equations of motion were integrated using a leap-frog algorithm with a timestep of 20 fs. The neighbour list was updated every 10 steps with a cutoff of 1.4 nm. The LJ and Coulomb potentials were smoothly shifted to 0 between 0 and 1.2 nm and between 0.9 and 1.2 nm, respectively, using the Gromacs shifting function. The center of mass motion was removed every step. Periodic boundary conditions were applied in all directions.

C. System composition and setup

In order to explore the behavior of a finite membrane patch at different conditions, the following systems were simulated:

- A flat bilayer made up of 928 neutral palmitoyl oleoyl glycerol POPC lipids plus 416 cholesterol molecules asymmetrically distributed in the two layers of the membrane (70% vs. 30%). The area per lipid of the two layers was not affected since the total amount of beads (POPC+CHOL) was kept constant in the two layers. It has been shown that A β monomers are stable inside a membrane of this specific composition.⁶⁹ The bilayer was inserted in a cubic water box (203 228 water beads, representing four times as many real water molecules) with a minimal distance of 5 nm from the lateral box boundaries and 10 nm along the normal direction. The electroneutral solution also contained 0.1 M sodium chloride.
- The same system as (a) but with a low concentration of A β peptides embedded into the bilayer (protein/lipid ratio 1/200). Additional sodium ions assured overall electroneutrality of the system.
- The same system as (a) but with a higher concentration of A β peptides embedded into the bilayer (protein/lipid ratio 1/24). Again, sodium ions were added to assure electroneutrality of the system.
- A flat bilayer made up of only 1300 POPC lipids embedded in a cubic water box (480 000 water beads). The electroneutral solution also contained 0.1 M sodium chloride.

All the patches in a–d were stabilized by short lipids at the rim. For each system, the simulations were repeated three times for better statistics by using different initial random velocities.

As a control, all of the above systems were additionally studied as infinite membrane patches (systems e–h). Additionally, system (a) was simulated in the presence of destabilizing short lipids at two different temperatures, 300 K (a') and 290 K (a''). Each system was simulated for 3 μs . The setup of system (a) is illustrated by snapshots shown in Figure 7.

FIG. 7. (a) Flat bilayer (cyan) with open borders embedded in a water solution (not represented) in the presence of short rim lipids (yellow). (b) Same system as (a) after removal of short lipids in excess.

FIG. 8. Gyration radius for finite lipid bilayers (without embedded peptides) simulated at different conditions: shown in orange, red, and blue are profiles of the patch stabilized at the rim by partially destabilizing short lipids at 300 K (systems a', simulated by starting at different initial velocities v_1, v_2); in blue, simulation of the previous system at the lower temperature of 290 K (a''); in brown and black, profiles of the patch stabilized at the rim by stabilizing short lipids (systems a, simulated at different initial velocities v_1, v_2).

Peptide aggregation was analyzed using a simple distance criterium: Peptides were defined to be clustered if the intermolecular distance was less than 0.5 nm (slightly larger than the bead size). In order to estimate the curvature stress induced on the membrane by the peptides, we calculated the gyration radius of the membrane.

D. Results

Finite membrane patches tend to close even in the absence of curvature-inducing peptides, i.e., to vesiculate to minimize the edge tension. Even membrane patches stabilized

with *standard* short-chain lipids (with $\epsilon_{ij} = 3.5 \text{ kJ mol}^{-1}$) vesiculate on the microsecond time scale (Fig. 8, *orange* and *red* lines). Here indeed, the short lipids partially mix with lipids of the patch, inducing a different increment of the area per lipid of the upper and lower layers. The same patch was partially stabilized at a decreased temperature of 290 K (system a'', *blue* line in Fig. 8), probably coupled to a decreased diffusion rate.

Here, we coated the edges by short-tail lipids with modified (*decreased*) Lennard-Jones interactions to the membrane lipids. Thereby, the edge tension is lowered such that the planarity of the membrane is maintained on the microsecond time scale in coarse-grained MD simulations (Fig. 8, *black* and *brown* lines). In our previous work,⁷⁰ we observed aggregation of A β peptides in a phospholipid membrane with a concentration dependent aggregate size. The A β aggregates were shown to have cone-shaped cross-sections, mainly due to the electrostatic repulsions between charges localized in the water-exposed region of the outer monolayer. Therefore, the area increase due to peptide insertion is not identical in the two leaflets of the bilayer, resulting in an increased local membrane curvature.

From the MD simulations performed here, it is evident that the response of the finite membrane patch to the curvature stress induced by the peptides is significantly stronger as compared to the case of the infinite bilayer, as theoretically predicted. The response is sensitive to the concentration of aggregating peptides: In the presence of a high concentration of peptides, the infinite bilayer is more bent than for a small peptide concentration (Figs. 9(a)–9(c)), as also seen for the gyration radius of the membrane patch in Fig. 10. For

FIG. 9. Snapshots at selected time frames (0, 0.2 μs , and after 3 μs) of an infinite bilayer (forced to planarity by periodic boundary conditions in the upper panels, (a), (b), and (c)) and a closing, finite lipid-protein bilayer with edge-stabilizing short lipids (dark grey) in the lower panels (a'), (b') and (c'); (a) and (a') bilayer (red) with high concentration of embedded A β peptides (yellow), (b) and (b') bilayer (blue) with a low concentration of embedded A β peptides (yellow), and (c) and (c') bilayer (grey) with no embedded peptides.

FIG. 10. Upper row: Gyration radius of lipids, and, lower part: average cluster size (number of aggregated peptides) for both the infinite membrane system (left side) and the finite membrane patch (right side) as a function of simulation time (the profile afterwards does not change in the remaining 1.5 μ s).

finite-sized membranes, a high peptide concentration even results in vesiculation (Fig. 9(a')). Interestingly, the cluster size, i.e., the number of aggregated peptides, is significantly decreased for the finite membrane patch (Fig. 10). In extended simulations, however, similar cluster sizes as for the infinite membranes were observed (data not shown), i.e., the diffusion of peptides in the strongly curved vesicle membrane is significantly reduced with respect to planar membranes.

IV. DISCUSSIONS AND CONCLUSIONS

We have theoretically investigated two basically identical systems (lipid membrane with axisymmetrically distributed hydrophobic inclusions) which differ for the outmost boundary conditions. For infinite membrane patches (as frequently used in MD simulations), the response of the bilayer to the impurities' *bending power* is often quite modest (see Sec. II C). In contrast, finite membrane systems that contain water-exposed edges stabilized by short-chain lipids show a more complex behavior characterized by two different regimes: (A) a planar unstable state, from which the system quickly evolves towards a stable vesicular state, and (B) a planar metastable state, with an energetic barrier to the stable vesicular state. Whether or not the system is unstable or metastable can be determined by employing the linear stability analysis developed in Sec. II D. We have proven that the stability increases with membrane bending rigidity, while it decreases with the size of the lipid patch, with the *bending power* of the impurities, and for large edge tensions (see Figs. 5 and 6). MD simulations fully confirmed the theoretical findings. Calculations performed at lower temperatures (290 K) show very stable lipid patches, while at increased temperature the planar arrangement becomes unstable. This behavior is related to the softening of the lipid bilayer in the more fluid phase where the bending rigidity may drop by up to an order of magnitude.^{71–76} Similarly, on increasing the size of the patch or modifying the chemical structure of the edge-active molecules, we favor unstable structures eventually leading to vesiculation as confirmed by our MD simulations. Such a general behavior has been tested for two different systems of finite bilayers that were differently stabilized

by short lipids at the boundaries and simulated at different temperatures.

The linear stability analysis developed in Sec. II D finds the boundaries between stable and unstable states but, unfortunately, it is unable to distinguish a real stable state from a metastable one. This goal can be achieved only by adding cubic and quartic terms in the formula for the energy of an open-edged lipid bilayer. The nonlinear differential equation resulting after minimization procedure does not generally have a closed analytical solution (a lucky case has recently been studied by Deserno and colleagues¹⁷), this fact requires the use of non-trivial numerical tools. An approximate solution has been obtained by us following the procedure suggested in Sec. II A. The most interesting result is that the height of the barrier between the planar metastable state and the final vesicular arrangement is proportional to the impurities' *bending power* (times a factor related to the patch bending rigidity and size), hence the *first passage time* for crossing the barrier is *exponentially* related to the *bending power*.

In summary, we have at hand two different methods to estimate the inclusions' *bending power*:

- A direct method that exploits infinite bilayers and provides an explicit estimate of membrane deformations by MD simulations. This method, however, generally suffers from a limited sensitivity and its response to the inclusions inducing curvature is related to the size of the simulation box.
- Instead, in this work we propose an indirect method that exploits stabilized water-exposed edges. It relies on the measurement of the residence time of a lipid bilayer in its metastable planar configuration, a datum that can be derived by MD simulations. The main advantage of our procedure should be the extreme exponential sensitivity of the bilayer response to the inclusions' *bending power*.

It must be kept in mind that procedure (b) can be applied only in a limited number of cases, that is, rigid membranes, small patches, and weak induced curvatures. Despite that, we were able to reach a good compromise through the stabilization of an open patch with in total 1200 lipids at coarse-grained level. The sketched procedure could also be applied in atomistic simulations. Here, restrictions due to the required computation time could be reduced by back-transformation of snapshots from coarse-grained simulations to atomistic detail.⁷⁷

In contrast, the standard approach (a) that makes use of PBCs for infinite membranes may yield extremely tiny deformations of the membrane that, in soft systems, could be masked by huge bending fluctuations, unless large simulation boxes are used. We additionally showed that infinite membrane simulation systems may yield a different aggregation behavior of transmembrane peptides, which is of particular importance, e.g., in the study of A β peptides used here as a model system.

The present work hints a possible alternative pathway to investigate the relevant coupling between membrane deformation and spatial distribution of curvature-inducing hosts. In order to reach a wide applicability, several issues must be further investigated. From the theoretical side, it is

mandatory to develop nonlinear models in order to explore the evolution of metastable states (post-buckling effects). Moreover, the constraint of an axisymmetric distribution of hosts can be removed. From the computational side, a detailed analysis of barrier crossing events^{78,79} combined with the computation of the associated potential of mean force will result in more quantitative predictions. Developments along these pathways are subject of ongoing research.

ACKNOWLEDGMENTS

This work was in part supported by the Emerging Fields Initiative *Synthetic Biology* at the Friedrich-Alexander Universität Erlangen-Nürnberg and the Deutsche Forschungsgemeinschaft (DFG) Research Training Group 1962 *Dynamic Interactions at Biological Membranes*. This work was also financially supported by a PRIN 2010/2011, Cod. prog.: 2010L96H3K.

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