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Vagelis A. Harmandaris[1] and Markus Deserno[2]
1. Max-Planck-Institute for Polymer Research Ackermannweg 10, D-55128 Mainz, Germany
2. Department of Physics, Carnegie Mellon University, PA 15213, Pittsburgh, USA
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Biological membranes are known to contain inhomogeneities that participate in intracellular transport processes. Membrane lipid phase coexistence affect these processes through the elastic properties at the phase boundaries. In this Letter we present a new hierarchical methodology for predicting the elastic constants of multicomponent membranes that combines coarse-grained simulations and numerical methods. Biphasic membranes have been simulated using a solvent-free coarse-grained bilayer model and the shape of the interfaces has been obtained. The results are compared with numerically resolved shape for various values of the elastic constants. With the proposed approach accurate values for all the elastic constants (bending moduli, saddle spay moduli, and line tension) of multi-component tethers can be obtained.

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Introduction.– Lipid bilayers, composed by amphiphilic molecules, are the basic structural component of biological cell membranes. In Nature lipid bilayers can be either the outer plasma membrane of cells or an interface separating different compartments inside the cell [1]. It has been suggested that the cellular membrane shows coexisting liquid phases, or domains called rafts [2], which play important role in cell signaling and traffic. While their existence in living cells is a matter of debate, micrometer-sized domains have been observed in giant unilamellar vesicles made from lipid mixtures [3]. These domains are also important for the intracellular traffic: budding and fusion take place in the presence of such domains with different elastic properties.

While the basic physical properties of homogeneous membranes are relatively well known the effects of the inhomogeneities within membranes are an active field of study. Mutiphase biological membranes have been studied both experimentally [5–7]. and theoretically [8–10, 12, 13]. In more detail experiments using high resolution flouroscense provide a correlation between domains decomposition and local membrane curvature the interface of biphasic membranes [5]. Using a complicated fitting procedure assuming ... More recently a method for obtaining the line tension between two different components by micropipete aspiration has been proposed [6]. Furthermore experiments show that fission happen mostly in phase separated vesicles, i.e. not in well mixed tethers. Numerical shapes of the interface have been also used to report upper bounds for the membrane parameters [9]. Very recently an analytical method to determine elastic parameters from experimental data has been developed by Semrau *et al.* assuming ... [10].

The difficulties associated with the calculation of the elastic constants of the multiphase tethers and accurately resolving the shape of the interface shows that this is still an open subject. Especially the calculation of Gaussian rigidity $\bar{\kappa}$ is an open and very difficult aspect. The reason is that according to Gauss-Bonnet theorem the total Gaussian curvature of a closed surface is constant, and equal to 2π times the Euler characteristic of the surface. $4\pi\bar{\kappa}g$ where g is the genus, the number of handles of he vesicle. For any fixed topology this term can be neglected. Therefore to get information about $\bar{\kappa}$ we need either to change topology, which in principle is very hard, or to change the boundary [11].

From all the above it is clear that any new information on how to calculate elastic constnts fo multiphase tethers and especially

$\bar{\kappa}$, either from experiments or from molecular simulation approaches, can be of great help. This is exactly the goal of the present work. In more detail, in this Letter we propose a new hierarchical approach for the study of multiphase membrane tubes that allows the accurate calculation of $\bar{\kappa}$ by studying the boundary between two phases. Our approach combines mesoscopic particle simulations and numerical schemes, and allows us to get accurately values for all the elastic constants (bending moduli, saddle spay moduli, and line tension) of biphasic membranes. First long coarse-grained (CG) simulations, using a recently proposed solvent-free model [14–16] of big biphasic tethers are performed. The simulations are analyzed and the shape of the model tethers is accurately resolved. Then the shape of biphasic tethers is calculated using a numerical solution for various values of the elastic constants. Through a fitting procedure the numerically obtained shape of the interface is matched with the shape of the modeled tethers. With this combined simulation-numerical procedure all the elastic constants in the two phases and in the interface, are accurately obtained. Here we focus on the study of the interface between two distinct liquid phases, each of which far away from the interface has a tubular shape, i.e. our model is a biphasic tubular membrane (tether).

Biphasic tethers can be analyzed on the continuum level using an elastic free energy model as introduced in the early 70's [4]. The respective Hamiltonian, can be written as a surface integral over the entire membrane and for a biphasic tether has the form:

$$E = \sum_{i=A,B} \int \mathrm{d}A \left\{ \frac{1}{2}\kappa_i (K - C_{0_i})^2 + \bar{\kappa_i} K_{\mathrm{G}} + \sigma_i \right\} + \oint_{d\alpha} \tau \mathrm{d}l. \tag{1}$$

where A and B correspond to the two phases and for each phase the free energy is integrated over its membrane area A_i. The extrinsic curvature $K = 1/R_1 + 1/R_2$ is the sum of the two local principal curvatures, and the Gaussian curvature $K_{\mathrm{G}} = 1/R_1 R_2$ is their product. The inverse length C_{0_i} indicates any spontaneous curvature which the bilayer might have, so the first term quadratically penalizes deviations of the local extrinsic curvature from C_{0_i}. The two moduli κ_i and $\bar{\kappa_i}$ belonging to the two quadratic curvature expressions are the *bending modulus* (or bending rigidity) and the *saddle splay modulus* (or Gaussian rigidity), of the respective phases. If the membrane has two identical leaflets, $C_{0_i} = 0$ by symmetry, a situation which does seldomly hold for biological membranes but very frequently for artificial lipid bilayers and vesicles. Thus any stress due to area differences between the leaflets or to an assymetry of the layers is quickly relaxed. Surface tensions σ_i are Lagrange multipliers introduced to ensure constant surface in each phase. Provided constant area per lipid, σ_i is constant in each of the phases. In the interface a positive line tension τ appears which involves the integral along the boundaries $d\alpha$. In equation 1 the effect of pressure is neglected.

Mesoscopic membrane simulation.– To illustrate our method, we have performed mesoscopic simulations of a coarse grained solvent-free lipid model recently developed in our group [14]. Roughly, lipids are represented by three consecutive beads of diameter σ (our unit length), with one end bead being hydrophilic and the two tail beads hydrophobic. The hydrophobicity is described as an effective attraction between the tail beads with a tuneable depth ϵ (our unit of energy) and range w_{c}. By properly choosing w_{c} and ϵ, a wide range of self-assembling fluid bilayer phases of different bending rigidities is obtained. More details can be found in Ref. [14].

Coarse-grained Molecular Dynamics simulations of model lipid tethers were performed using the ESPResSo package [18].

	# lipids	$L_z(\sigma)$	$w_c^{AA}(\epsilon)$	$w_c^{BB}(\epsilon)$	$w_c^{BB}(\epsilon)$	$k_A(k_BT)$	$k_B(k_BT)$	$\tau(k_BT/\sigma)$	$\bar{\kappa_\alpha} - \bar{\kappa_\beta}(k_BT)$
1	40000	400	1.6	1.6	1.5	12	12	0.95 ± 0.05	0
2	60000	500	1.6	1.5	1.45	12	8	0.77 ± 0.10	-4.1
3	80000	500	1.6	1.5	1.45	12	8	0.75 ± 0.10	-4.2

TABLE I: List of model tethers studied and values of their material parameters obtained from our methodology.

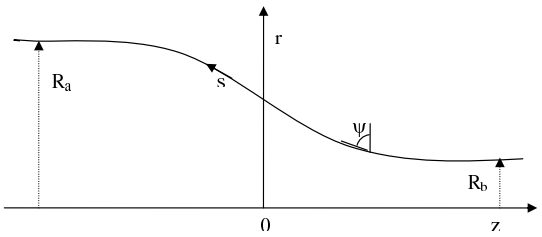

FIG. 1: Geometry sketch of a periodic biphasic tether and of the interface with z-axis being the axis of the symmetry of the tether.

The systems studied are summarized in Table I. Different components are modeled using different values for the range of the effective hydrophobic attraction w_c^{ij} between components i and j (here $i,,j = A, B$). All simulations were performed under canonical (NVT) conditions, using a Langevin thermostat with friction constant $\Gamma = 1.0\,\tau^{-1}$ to keep the temperature constant. Within a rectangular box with dimensions $L_x = L_y$ and L_z, using periodic boundary conditions in all directions, a cylindrical periodic biphasic membrane spanning the z-direction was initially set up with. As it was also shown elsewhere phase separation in our model tethers occurs in a much faster time scale than the development of the interface (or fussion) [14]. That is in agreement with experimental observations [5]. Therefore we are performing the CG MD simulations starting from a biphasic tether with a "$A - B - A$" structure, i.e. our modeled tethers are periodic with a B bulk phase lying between two A bulk phases. The radius R^{setup_i} chosen in such a way that the area per lipid in both leaflets corresponded to the one for a flat tensionless bilayer for each phase i [14, 15]. For the integration, a time step of $\Delta t = 0.01\,\tau$ was used for most of the systems, while in some cases we needed a smaller time step of $\Delta t = 0.005\,\tau$ in order to obtain accurate results.

Numerical Scheme.– The membrane can be parameterized by the arc length s along the contour (See Figure 1). The contour is described by the functions $r(s)$, $z(s)$ and $\psi(s)$, where r is the distance of the local axis of the rotational symmetry (local tube radius), z is the coordinate along the axis and ψ is the tilt angle of the contour.

The variational derivation of the shape equation of the surface is for each phase [8]:

$$\dddot{\psi} = -\frac{\dot{\psi}^3}{2} - \frac{2cos(\psi)}{r}\ddot{\psi} + \frac{3sin(\psi)}{2r}\dot{\psi}^2 + \frac{3cos(\psi)^2 - 1}{2r^2}\dot{\psi} \tag{2a}$$

$$-\frac{cos(\psi)^2 + 1}{2r^3}sin(\psi) + \frac{\sigma_i}{\kappa_i}\dot{\psi} + \frac{\sigma_i}{\kappa_i}\frac{sin(\psi)}{r} \tag{2b}$$

$$\dot{r} = cos(\psi), \dot{z} = -sin(\psi). \tag{2c}$$

where all the overdots denote derivatives with respect to s. Far away from the interface homogeneous cylindrical tether solution is recovered with $\psi = \pi/2$ and $R_i = (\kappa_i/2\sigma_i)^{1/2}$. Due to mechanical equilibrium axial forces at both bulk phases should be equal, i.e. $F^z_\alpha = F^z_\beta$ with $F^z_i = 2\pi\kappa_i/R_i$ [15]. This leads to $\sigma_\alpha/\sigma_\beta = \kappa_\alpha/\kappa_\beta$. Four boundary conditions should be satisfied at the interface (which assumed to be at zero) [8]. Two that impose continuity in r and ψ:

$$\psi(+\epsilon) = \psi(-\epsilon), r(+\epsilon) = r(-\epsilon). \tag{3a}$$

and two due to mechanical equilibrium at the interface:

$$\kappa_\alpha\dot{\psi}(+\epsilon) - \kappa_\beta\dot{\psi}(-\epsilon) = (\Delta\kappa + \Delta\bar{\kappa})\frac{sin(\psi(0))}{r(0)} \tag{4a}$$

$$\kappa_\alpha\ddot{\psi}(+\epsilon) - \kappa_\beta\ddot{\psi}(-\epsilon) = \tau\frac{sin(\psi(0))}{r(0)} \tag{4b}$$

$$+(2\Delta\kappa + \Delta\bar{\kappa})\frac{sin(\psi(0))cos(\psi(0))}{r(0)^2} \tag{4c}$$

with $\Delta\kappa = \kappa_\alpha - \kappa_\beta$ and $\Delta\bar{\kappa} = \bar{\kappa_\alpha} - \bar{\kappa_\beta}$. Domain boundary located at $s = 0$. As we can see in the interface only the difference between the values of $\bar{\kappa_i}$ of the two components, and not their actual values, play a role. Therefore we can calculate values κ_i separately but only the difference in $\bar{\kappa_i}$. The set of the above equations, Eqs. 2-4, can be solved using a shooting to a fitting point method [17, 19]. The method essentially involves shooting from both bulk phases to the junction point (at zero) where the four boundary conditions should be satisfied. The fourth-order dimensional type of shooting involves a global search algorithm. More details of the numerical procedure can be found elsewhere [17].

Results.– Our goal is to study the interface of the biphasic model tethers and to obtain accurately the elastic constants of each phase, using the shapes derived from the simulations and the numerical analysis of the shape. The overall methodology can be briefly described as following: first model biphasic tethers are simulated and he shape at the interface is accurately resolved. Then the shape of the biphasic tethers is numerically obtained. The solution involves the calculation of 5 elastic constants: the two bending rigidities κ_i, the two Gaussian rigidities $\bar{\kappa_i}$ and the interfacial tension τ. The bending rigidity of each component can be calculated using a recently proposed method through simulations of stretched tethers [15]. The tensile force needed to hold a membrane tether is proportional to membrane modulus. Thus, performing simulations of the model single component tethers and measuring the required force allows us to calculate accurately κ_A and κ_B (see Table I).

The line tension between the two phases can also be calculated independently from simulations of biphasic flat bilayers. In more detail for flat bilayers interfacial line tension is obtained as the difference between the total parallel and perpendicular stress tensor. The stress tensor, σ_{ij}, is obtained using the virial theorem. Results about the values of the line tension τ for the systems studied here are shown in Table I. This leave us with only one unknown parameter, i.e. the difference between the two Gaussian rigidities $\Delta\bar{\kappa}$. This can be calculated through a single parameter fitting of the numerical solution to the shape obtained from the CG simulations [17]. Therefore in our methodology a clean and accurate way to obtain all the relevant elastic constants is proposed.

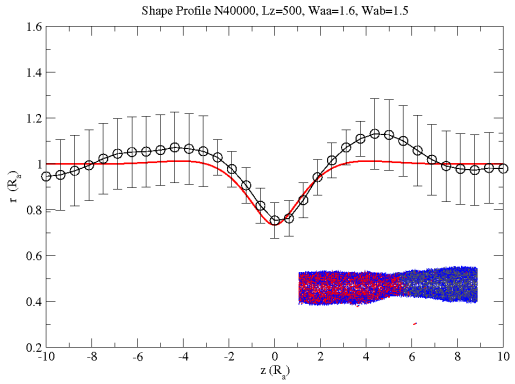

FIG. 2: Shape of the interface for a biphasic tether with "A" and "B" phases similar and with a positive interfacial tension. In the inset snapshot of a CG biphasic tether with $40\,000$ lipids is shown. Note that the aspect ratio of the tethers is very big since r and z are measures in different units.

As a consistency check of the proposed methodology we first study systems with identical A and B phases but with a non zero interfacial line tension. This "unphysical" situation can be easily modeled by properly choosing the values of the parameters w_c^{ij} (see Table I), i.e. $w_{AA} = w_{BB} > w_{AB}$. For such a system the radius of the two bulk phases is determined from the two bending rigidities, κ_A and κ_B, whereas the interphase is determined solely from the value of the line tension τ, since both $\Delta\kappa$ and $\Delta\bar{\kappa}$ are 0. As mentioned above, κ_A, κ_B and τ can be calculated independently from CG simulations of single component tethers and of two component bilayers respectively. These values can be used in for obtaining the numerical shape of the two-component tethers. Thus, no fitting needed in order to compare the numerical solution with the shape obtained from the mesosocpic simulations of the periodic two-component model tethers.

Then we need to calculate the radius as a function of diastance, $r(z)$. For this we use the midplane between the two monolayers to denote the average radius $\langle r \rangle$. It is determined by first identifying the axis, next finding the average distance of the second tail bead of the outer leaflet to this axis, R_{out}, and the equivalent for the inner leaflet, r_{in}. We then take $\langle r \rangle = \frac{1}{2}\langle r_{\text{out}} + r_{\text{in}} \rangle$, where the average is taken during long production runs typically extending over 10000-20000 τ. Errors are determined via a blocking analysis. Average radius is determined locally in slices xyz. Location of the boundary is traced while it diffuses along the direction of the tether. Inset in Figure 2 shows a typical snapshot of two-component cylindrical vesicles (tethers) from the CG simulations. Notice that while fluctuations are clearly visible, they are fairly weak, *i. e.*, the vesicle is to a very good approximation cylindrical with a clear interphase between two bulk phases. The shape of the interface for the above systems is analyzed and shown in Figure 2 with circles.

Numerical solution of the shape equation is also shown in Figure 2 (solid line). The results show a remarkable qualitative and quantitative agreement between the simulated profile and the numerical solution. This is of particular importance if we consider that shape profiles are directly compared *without any adjustable parameter*. The agreement works as a consistency check for both the numerical solution and the CG simulations.

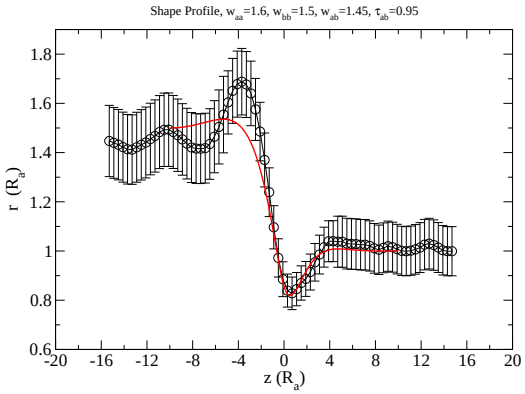

FIG. 3: Shape of the interface for a biphasic tether with "A" and "B" phases being different. In the inset snapshot of a CG biphasic tether with 60 000 lipids is shown.

Next we study more realistic two-component model tethers with different lipid phases A and B. Assuming that $\bar{\kappa_i} = c\kappa_i$ with $i = A, B$ the constant c can be calculated. Stability arguments impose that $-2 < c < 0$ [**?**]. A more constrained study, which assumes that a compression of the hydrocarbon chains in one direction increase the splay in th orthogonal direction, founds that $-1 < c < 0$ [20]. This provides a direct way to calculate accurately the difference between the Gaussian rigidities of the two components.

As a final point we would like to address the issue of finite size effects. For this reason systems with different radius were studied ...

Discussion. − We have presented a new approach for studying multiphase tethers which combines numerical methods and coarse-grained simulations.

All the values of the elastic constant parameters are reported in Table I.

We believe that the overall methodology provides a rigorous way to obtain the elastic constants characterizing the interface of a multiphase membrane. By performing coarse-grained simulations the shape of a multiphase membrane tether can be obtained. The interface then can be fitted to a numerical obtained one and the difference between the Gaussian rigidities of the two phases can be obtained.

A further advantage of the proposed method is that the shape of the interface of the model multiphaee tether can be accurately obtained.

Acknowledgments

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[1] H. Lodish, A. Berk, L. Zirpursky, P. Matsurada, D. Baltimore, and J. Darnell, *Molecular Cell Biology*, (Freemen and Company, New York, 2000).

[2] K. Simons and E. Ikonen, Nature **387**, 569 (1997); R.G.W. Andersen and K. Jacobson, Science, **296**, 569 (2002); S. Munro, Cell, **115**, 377 (2003); M. Edidin, Ann. Rev. Biophys. Biomol. Struct. **32**,2 257 (2003).

[3] C. Dietrich *et al.*, Biophys. J. **80**, 1417 (2001);

[4] P.B. Canham, J. Theoret. Biol. **26**, 61 (1970); W. Helfrich, Z. Naturforsch. C **28**, 693 (1973); E. Evans, Biophys. J. **14**, 923 (1974).

[5] T. Baumgart, S.T. Hess, and W.W. Webb, Nature **425**, 821 (2003); T. Baumgart, S. Das, W.W. Webb, and J.T. Jenkins, Biophys. J. **89**, 1067 (2005).

[6] A. Tian, C. Johnson, W. Wang, and T. Baumgart, Phys. Rev. Lett. **98**, 208102 (2007).

[7] D.P. Siegel and M.M. Kozlov, Biophys. J. **87**, 366 (2004).

[8] F. Julicher and R. Lipowsky, Phys. Rev. E. **53**, 2670 (1996).

[9] J.M. Allain, C. Storm, A. Roux, M. Ben Amar, and J-F. Joanny, Phys. Rev. Lett. **93**, 158104 (2004); J.M. Allain and M. Ben Amar, Eur. Phys. J. **20**, 409 (2006); F. Campelo, J.M. Allain, and M. Ben Amar, Eur. Phys. Lett. **77**, 38006 (2007).

[10] S. Semrau, T. Idema, L. Holtzer, T. Schmidt, and C. Storm, Phys. Rev. Lett. **100**, 088101, (2008).

[11] M.P. do Carno, *Differential Geometry of Curves and Surfaces*, (Prentice Hall, New Jersey, 1976).

[12] J. Liu, M. Kaksonen, D.G. Drubin, and G. Oster, PNAS **27**, 10277 (2006).

[13] X. Wang and Q. Du, J. Math. Biol. **56**, 347, (2008).

[14] I.R. Cooke, M. Deserno, and K. Kremer, Phys. Rev. E **72**, 011506 (2005).; I.R. Cooke and M. Deserno, J. Chem. Phys. **123**, 224710 (2005).

[15] V.A. Harmandaris and M. Deserno, J. Chem. Phys. **125**, 204905 (2006).

[16] B. Reynolds, G. Illya, V.A. Harmandaris, M.M. Mller, K. Kremer, M. Deserno, Nature **447**, 461-464, (2007).

[17] V.A. Harmandaris and M. Deserno, Unpublished results.

[18] H.J. Limbach, A. Arnold, B.A. Mann, and C. Holm, Comp. Phys. Comm **174**, 704 (2006).

[19] W.H. Press, S.A. Teukolsky, W.T. Vetterling, B.P. Flannery, *Numerical Recipes*, (Campridge University Press, 2007) 3rd ed.

[20] R.H. Templer, B.j. Khoo, and J.M. Seddon, Langmuir **14**, 7427 (1998).