Interaction and Adhesion of Vesicles: Coupling between Attractive Interactions and Bilayer Membrane Flexibility

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Interaction and Adhesion of Vesicles: Coupling between Attractive Interactions and Bilayer Membrane Flexibility

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A simple model for vesicle interaction and adhesion, due to nonspecific colloidal forces, is presented. It is shown that possible coupling with the deformations of the bilayer lipid membrane may substantially enhance the effect of the attractive forces. Thus, the presence of a water core in the vesicle interior decreases the strength and the range of the van der Waals attraction, but the deformation of the lipid bilayers may compensate this effect to a certain extent. Therefore, the total attractive energy (in the absence of specific interactions or other sources of attraction like depletion forces, etc.) could be due to coupling between the van der Waals and bending elasticity forces. The presence of excess line energy may lead to the appearance of a barrier in the interactions. As a result, a complex energy versus distance profile could be observed.

1. Introduction

Bilayer lipid vesicles are widely used for model studies of the interaction between biological membranes. Phenomena like structure formation from aggregates of biological cells and cell fusion involve membrane adhesion as an important step. Membrane fusion itself is of great practical importance for applications such as targeted drug delivery, using vesicles. A lot of attention is occupied with the role of the site-specific (ligand−receptor) type of interactions.[1] Still, it has been shown that the addition of calcium ions in the suspension can readily induce anionic vesicle aggregation and fusion.[2] The loss of aggregation stability, due to the presence of ions with charge opposite to that of the suspended particles, is well-known in colloid science and has been explained successfully by the Derjaguin−Landau−Verwey−Overbeek (DLVO) theory.[3−9] This theory, for example, gave a quantitative interpretation the effect of the counterion valency on the suspension destabilization, which was established experimentally by Schulze[10] and Hardy.[11] That is why results such as those reported in ref 2 make us believe that nonspecific colloidal interactions may also be important for the overall behavior and stability of vesicular systems. In fact (as pointed out in ref 9, p 284) the attraction between lipid bilayers is well described by the van der Waals force when the hydrophilic headgroups completely screen the hydrocarbon chains. The purpose of the present study is to calculate the magnitude and examine the role of these forces. The specific features of the vesicles, like the presence of a water

core and the bending flexibility of the lipid bilayers, are taken into account. The water core decreases substantially the van der Waals attraction, but as we show below, this could be compensated by the deformability of the vesicle walls. In fact the coupling between van der Waals attraction and bilayer deformation may lead alone to intervesicle adhesion. The repulsive surface forces (e.g. electrostatic, steric, hydration, etc.) will not be accounted for explicitly, to maintain clarity of the presentation and to emphasize the effects we are after. Instead, a minimal distance of lipid bilayer approach will be used as a parameter, responsible for the interbilayer repulsions present.

Every attractive force may couple with the membrane deformability in order to produce a stronger interaction (compared to a reference nondeformable case). Our choice to use van der Waals interactions in this study is due to the following reasons:

(i) While many types of surface forces (i.e. depletion, hydrophobic, etc.) may exceed the magnitude of the van der Waals interaction (*at given separation*), their presence or absence depends on the particular system under consideration. van der Waals interactions, on the other hand, are always present, no matter what type is the colloidal system. In fact there is evidence that for the case of lipid bilayers van der Waals attractions are the relevant ones, provided that the hydrophilic headgroups are screening the hydrophobic tails from contact with the water surroundings (see ref 9, p 284, and references therein).

(ii) The effect of the coupling between attraction and membrane flexibility is more clearly illustrated when a relatively weak (in some cases) interaction is used.

(iii) van der Waals interactions are divergent at zero separation, which means that they could be very strong at sufficiently small distances, of course if they are physically accessible.

There are certainly many cases in which one should take into account other possible interactions. Their incorporation into the present approach is straightforward.

Only unilamellar vesicles will be considered. The basic concept of how the vesicles approach and adhere, which we use throughout the present study, is illustrated in Figure 1. The particles are spherical when separated at large distances. However, when they approach each other (e.g. because of the van der Waals attraction), they may

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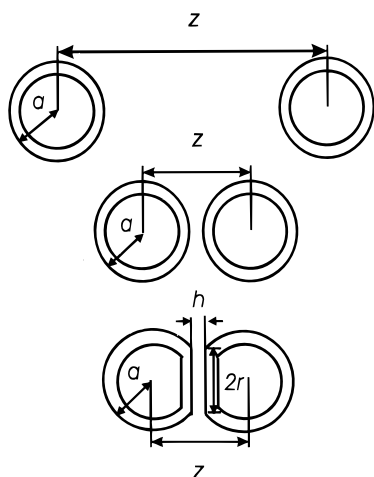(11) Hardy, W. B. *Proc. R. Soc. London* **1900**, *66*, 110.

Figure 1. Sketch of two interacting vesicles.

acquire the shape of truncated spheres separated by a water layer at sufficiently small distances, which are determined by the repulsive surface forces (see also ref 1). The balance of all the energy contributions may lead to a stable configuration of two adhered vesicles, as depicted in Figure 1.

The paper is organized as follows: in the next section we discuss the van der Waals attraction between vesicles, section 3 deals with effects stemming from the bending flexibility of the lipid bilayers, forming the vesicle walls, section 4 shows how the combined action of the van der Waals attraction and bilayer deformability affects the adhesive behavior of the vesicles, and section 5 summarizes the concluding remarks.

2. van der Waals Interactions between Vesicles

The van der Waals interaction for two colloidal spheres was calculated by Hamaker,[12] assuming pairwise additivity between the molecules in the macroscopic bodies and neglecting the effect of electromagnetic retardation, which effectively decreases the magnitude and range of the force.[13] The Hamaker method leads to an exact (within the framework of the above assumptions) solution for two interacting spheres. However, hereafter we will be interested only in the case of small separations, since (as shown below) all the substantial interactions take place when the distance between the vesicle centers is about, and even sometimes less than, the sum of their radii. Thus for two equal spheres at small separation, we have the following simple expression for the van der Waals interactions[7,8]

$$U_{VW} = -\frac{A}{12}\left[\frac{a}{h} + 2\ln\left(\frac{h}{a}\right) + \frac{3}{4}\right] \quad (1.1)$$

where A is the Hamaker constant,[12] a is the colloidal particle, radius and h is the distance between the surfaces of the two spheres. The Hamaker constant has dimensions of energy and depends on the material properties of the colloids and the surrounding medium. It is multiplied by a function, which accounts for the geometry of the interacting objects. If the particles are not hard enough to resist deformation (e.g. droplets, vesicles, etc.), they may deform and acquire roughly the shape of truncated spheres. This geometry also allows for an exact solution

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to be derived,[14,15] but again we are interested in small separation where the van der Waals energy is

$$U_{VW} = -\frac{A}{12}\left[\frac{a}{h} + 2\ln\left(\frac{h}{a}\right) + \frac{3}{4} + \frac{r^2}{h^2} - \frac{2r^2}{ah}\right] \quad (1.2)$$

The last two terms are due to the formation of a planar layer of solvent between the flattened bilayers, with radius r and thickness h (see Figure 1).

The case of inhomogeneous particles is more complicated, since the different materials are characterized by different Hamaker constants. For spherical particles with a layered structure the van der Waals interaction is given by[16]

$$U_{VW} = -\sum_{i=1}^{N}\sum_{j=1}^{M}A(i,j)\,H(x_{ij}) \quad (1.3)$$

where

$$A(i,j) = A_{i,j} - A_{i,j-1} - A_{i-1,j} + A_{i-1,j-1} \quad (1.4)$$

and $A_{i,j}$ is the Hamaker constant of interaction between phases i and j through vacuum. $H(x_{i,j})$ is a geometrical function, taking into account the specific shape and distance between the different layers. For the particular case of deformed vesicles, like those in Figure 1, eqs 3 and 4 give

$$U_{VW} = -\frac{A_{OWO}}{12}\left[\frac{a}{h} + 2\ln\left(\frac{h}{a}\right) + \frac{3}{4} + \frac{r^2}{h^2} - \frac{2r^2}{ah}\right] -$$
$$\frac{A_{WOW}}{12}\left[\frac{a-\delta}{h+2\delta} + 2\ln\left(\frac{h+2\delta}{a-\delta}\right) + \frac{3}{4} + \frac{r^2}{(h+2\delta)^2} -\right.$$
$$\left.\frac{2r^2}{(a-\delta)(h+2\delta)}\right] + \frac{A_{WOW}}{6}\left[\frac{a-\delta}{h+\delta} + 2\ln\left(\frac{h+\delta}{a-\delta}\right) +\right.$$
$$\frac{3}{4} + \frac{r^2}{(h+\delta)^2} - \frac{r^2}{(a-\delta)(h+\delta)}\left(\frac{15}{8} + \frac{h+3\delta}{8(h+\delta)}\right) +$$
$$\left.\frac{\delta}{2(h+\delta)}\right] \quad (1.5)$$

The Hamaker constants A_{OWO} and A_{WOW} are for oil phases (lipid bilayer of thickness δ in our case) interacting across the water layer and for water phases interacting across the oil layer, respectively. The corresponding geometrical functions were derived originally for calculating the van der Waals attraction between protein-stabilized emulsion droplets.[17] The first line of eq 1.5 is identical to eq 1.2, which holds for homogeneous particles (e.g. oil droplets interacting in water medium), while the following two lines represent a correction, due to the presence of a water core inside the vesicles. The replacement of part of the particle oil interior with water decreases both the magnitude and range of the interaction, which is shown in Figure 2. The lower curve is for homogeneous oil spheres with the Hamaker constant $A_{OWO} = 4 \times 10^{-21}$ J and is calculated by eq 1.2. The upper curve is for spherical vesicles of the same radius and Hamaker constants $A_{OWO} = A_{WOW} = 4 \times 10^{-21}$ J. The bilayer thickness is chosen to be $\delta = 0.01a$. No deformation is assumed to be present in this case. Hence, it could be expected that the van der Waals interaction would be less responsible for the

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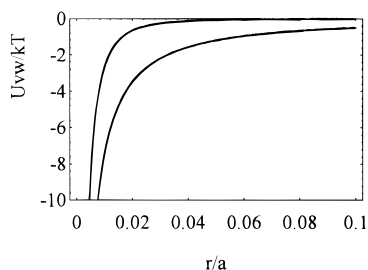(17) Denkov, N. D.; Cholakova, S. To be published.

Figure 2. van der Waals attractive energy between two spheres of radii a versus the distance between their surfaces h/a. The lower curve is for a homogeneous oil sphere with Hamaker constant $A_{OWO} = 4 \times 10^{-21}$ J. The upper curve is for a vesicle with the membrane (oil phase) thickness $\delta = 0.01a$. $A_{OWO} = A_{WOW}$.

aggregation and destabilization of the vesicle suspension compared to the case of homogeneous colloidal particles, with the same Hamaker constant as that of the bilayers interacting across water.

3. Contribution of the Bilayer Bending and Extension Energy

The bending energy contributes to the deformation resistance of fluid particles and hence to the total energy of interaction.[14,15,18,19] It depends in general on the bending elasticity constant k_C and the radius of spontaneous curvature. It is proven that the latter is zero for symmetric lipid bilayers.[20] Then the surface energy density for flattening of two spherical vesicles (as shown in Figure 1) is $-4k_C/a^2$, with k_C being the bending elasticity constant (see refs 15, 18, and 19). It is favorable, since the lipid bilayer prefers to be flat instead of having any nonzero curvature. Hence, this energy contribution facilitates the adhesion between vesicles (see Figure 1). It also may increase the tendency for fluctuation deformations of an isolated vesicle. The boundary between the flattened bilayer part and the spherical vicinity of the vesicle, however, is a region with curvature greater than that of an unperturbed spherical vesicle. Such increased local curvature is energetically unfavorable (the radius of preferred curvature is zero). This positive energy contribution is proportional to the perimeter of the plane parallel region, formed between the two deformed vesicles (see Figure 1). The total (curvature related) energy, due to the flattening of parts of the two interacting vesicles, could be written as

$$U_C = -4\pi k_C \frac{r^2}{a^2} + 2\pi \tilde{\chi} \frac{r}{a} \qquad (2.1)$$

where $\tilde{\chi} = \chi a$ is the line energy excess at the flattened bilayer periphery.

Finally one should take into account the energy related to the bilayer extension upon deformation. Bilayer lipid membranes are usually considered to have zero surface tension, but the extension could be opposed by the stretching energy, which we write in the form[14,21]

$$U_S = E_G \frac{\pi a^2}{64} \frac{r^8}{a^8} \qquad (2.2)$$

E_G is the Gibbs' elasticity. This energy contribution is

positive and prevents the deformation and therefore adhesion between the vesicles.

4. Results and Discussion

In this section we analyze how the combination of the different energy contributions, considered above, relates to the total interaction and adhesion between vesicles. All distances and sizes, used in the calculations below, are scaled with the vesicle radius a. The only restriction on the vesicle size a is that it should be much greater than the bilayer thickness δ and the interbilayer distance h. For example, the calculations presented below could be relevant to vesicles with radius $a = 500$ nm, bilayer thickness $\delta = 5$ nm, and the interbilayer separation varying between 5 and 10 nm. We do not account explicitly for repulsive interactions (electrostatic, steric, hydration, etc.). More details about these interactions could be found in ref 22. These effects are introduced by defining the minimal distance of bilayer approach h in our calculations. Note that this is an input parameter, effectively defining the magnitude and range of the repulsive interactions. In general the following scenario is assumed: as the vesicles approach and reach the minimal interbilayer distance (where the van der Waals attraction between nondeformed vesicles is balanced by the repulsive force), they may start to deform. At the beginning of the deformation the unfavorable line energy dominates and therefore the energy increases. However, the line energy increases linearly with the radius r of the deformed region, while the favorable bending contribution due to flattening and the attractive van der Waals energy depend on r^2 and become more substantial with the extent of the deformation. Thus, an energy barrier is formed after which the overall attraction and vesicle deformation increase. The latter cannot proceed to infinity because the vesicle volume is assumed to be constant and the lipid bilayer extension is restricted by the Gibbs elasticity. This leads to an increase of the energy after passing through a minimum. By varying different parameters, one may switch from an adhesive to a nonadhesive regime, which is illustrated below.

Figure 3 presents some calculations for the interaction between vesicles as a function of the radius of the deformed region (see Figure 1). All the energy contributions due to van der Waals attraction (see eqs 1.3−1.5) and bilayer flexibility (eqs 2.1 and 2.2) are included. The different curves in Figure 3a are for different values of the bending elasticity constant k_C. The bending constant is greatest for the lowest curve ($k_C = 30$), while for the highest it is zero. The intermediate curves show how the interaction energy evolves with the bending rigidity (see ref 22 for the typical range of values for the bending elasticity constant k_C). The reason for this attractive well which develops with the increase of the bending elastic constant is that, as discussed above, the radius of spontaneous curvature of the bilayer is zero and the flat configuration has the lowest bending energy. The van der Waals attraction is also present, but it can be seen that for low bending rigidity it cannot alone lead to adhesion, at least for these Hamaker constants,[23] bilayer thicknesses, and parameters E_G and χ. The flattening of the vesicles, on the other hand, increases the van der Waals attraction,

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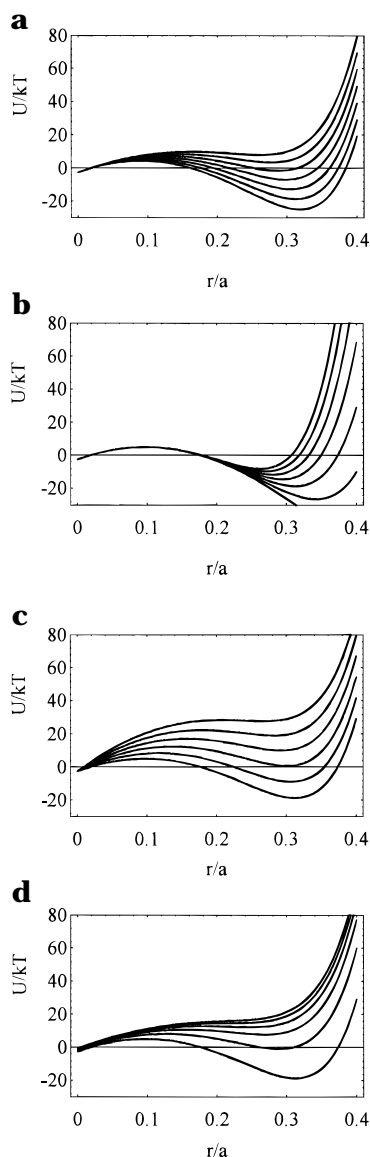(19) Petsev, D. N.; Linse, P. *Phys. Rev.* **1997**, *E55*, 586.
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a

b

c

d

Figure 3. Total energy of adhesion (see eqs 1.5, 2.1, and 2.2] between two vesicles of radii a, separated by the distance $h = 0.01a$, versus the radius of the deformed plane parallel region. $A_{OWO} = A_{WOW} = 4 \times 10^{-21}$ J, and $\delta = 0.01a$. (a) $E_G = 50$ mN/m, and $\chi a = 25kT$. The different curves correspond to different bending constants k_C, starting at the top: $0kT$, $5kT$, $10kT$, $15kT$, $20kT$, $25kT$, and $30kT$. (b) $k_C = 25kT$, and $\chi a = 25kT$. The different curves correspond to different Gibbs' elasticity constants E_G, starting from the bottom: 10, 30, 50, 70, 90, 110, and 130 mN/m. (c) $k_C = 25kT$, and $E_G = 50$ mN/m. The different curves correspond to different line energies χa, starting from the bottom: $25kT$, $30kT$, $35kT$, $40kT$, $45kT$, and $50kT$. (d) $k_C = 25kT$, $E_G = 50$ mN/m, and $\chi a = 25$ kT. The different curves are for different magnitudes of the repulsive interactions, starting from the bottom: $h = 0.01a$, $0.012a$, $0.014a$, $0.016a$, $0.018a$, and $0.02a$.

which is geometrically sensitive and is stronger between parallel flat bilayers than between curved ones. The excess line energy is assumed to remain constant with varying bending rigidity, which is questionable. The rigorous calculation of this energy, although important, is out of the scope of the present consideration, and we consider it as if it is an independent quantity.

In Figure 3b the bending elasticity constant is kept constant, $k_C = 25kT$, but the Gibbs elasticity is varied from $E_G = 10$ mN/m (lowest curve) to $E_G = 130$ mN/m (highest curve). Again the range was chosen to be within reasonable limits.[24] If the bilayer is readily expandable,
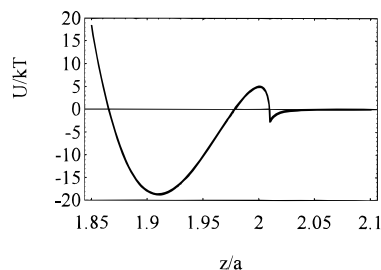

Figure 4. Interaction energy (see eqs 1.5, 2.1, and 2.2] as a function of the distance between the vesicle mass centers z. $k_C = 25kT$, $E_G = 50$ mN/m, $\chi a = 25kT$, and $h = 0.01a$. $A_{OWO} = A_{WOW} = 4 \times 10^{-21}$ J, and $\delta = 0.01a$.

the deformation could be practically unlimited. There is a minimum in energy, however, for the entire set of Gibbs elastic constants we used.

Figure 3c shows the cases of different line energies, while the other parameters are constant. The bending elasticity constant is $k_C = 25kT$, and the Gibbs elasticity is $E_G = 50$ mN/m. The choice of range for varying the parameter χ is based on ref 25. As can be expected, the increase of the line energy makes the vesicles less prone to deformation and therefore to adhesion. As we also mentioned above, contrary to the van der Waals energy, the bending contribution does not need a combination of two particles in order to produce deformation (e.g. flattening) of the bilayer. Hence, a single vesicle may experience local shape fluctuation, leading to flattening, which is favored by the first term in eq 2.1 and opposed by the second one as well as by 2.2. The balance of these contributions determines whether the vesicles will remain preferably spherical or will undergo shape fluctuations. When two vesicles approach, the bilayer flexibility couples with the van der Waals attraction to give the overall adhesive energy.

Figure 3d shows the importance of the repulsive interactions on the adhesive behavior of the vesicles. As might be expected, the repulsion has a very strong effect by competing with the attractive van der Waals forces. As the latter decrease strongly with distance, even a slight increase of h may prevent vesicle adhesion. Thus for $h = 0.01a$ there is a well-defined attractive minimum of $-18.67kT$, but at $h = 0.012a$ it is only $-0.89kT$. Further increase of h leads to complete disappearance of the attractive well and the adhesion (the repulsion becomes dominant). Hence, by tuning the repulsive interactions, one may control the adhesive properties of the vesicles. This could be easily achieved in the case of ionic vesicles by varying the ionic strength or in the nonionic case by changing the temperature.

All the parts of Figure 3 show that a negative (attractive) interaction energy could be observed at $r \to 0$, that is, without significant bilayer deformation. This attraction is due to the van der Waals forces for nondeformed vesicles (see Figure 2), and its value, for the minimal distance $h/a = 0.01$ used, is $-2.67kT$. This energy does not depend on the flexibility properties of the vesicle lipid bilayer and for some cases could be the only option for attraction. Hence, Figure 3 shows how, by changing the membrane flexibility and stretching properties, the system switches from weak attraction without deformation to adhesion with an energy of the order of tens of kT.

Figure 4 represents the dependence of the overall interaction energy (see eqs 1.5, 2.1, and 2.2 on the distance

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between the vesicle centers z. The bending elastic constant is $k_C = 25kT$, the Gibbs elasticity is $E_G = 50$ mN/m, and the line energy is $\chi a = 25kT$. The minimal bilayer distance is assumed to be $h = 5$ nm. For $z > 2a$ the vesicles start to attract each other due only to the van der Waals force (for nondeformed particles). At the minimal bilayer distance ($z/a = 2.01$) the attractive energy is $-2.67kT$ but no further approach of the bilayers is possible due to the repulsion determined by h. However, the vesicle centers can approach further, which is accompanied by deformation. The initial stage of the deformation is unfavorable, because of the line effects, and the energy sharply increases. With the decrease of the distance z/a, the combined action of the favorable bending and van der Waals energies becomes dominant and the system arrives again in an attractive region. Finally, the minimum is formed when the Gibbs elasticity takes over and prevents further deformation. Comparing the two minima, before and after deformation, we may conclude that the formation of doublets of deformed vesicles, adhered to each other, is more likely than sticking as nondeformed spheres, for the parameters in this particular case. Also the attractive well before deformation would be additionally decreased if repulsions of range greater than h are present (e.g. electrostatics at low salt concentrations). Other interactions known to enhance the interbilayer repulsion are the so-called undulation forces,[22] that are also coupled to the membrane flexibility. These forces, however, are related to a different mode of bilayer deformation[22] (normal undulations of the membrane) than the one considered in this study (formation of truncated spheres). Besides, it was found[22] that their most appreciable contribution is at interbilayer distances below 1.7 nm and decays exponentially at larger separations. The length scale of the repulsions in the present calculations starts at 5 nm. Still, the possible contribution of the undulation forces is effectively taken into account by the parameter h. Besides the favorable bending energy contribution (due to flattening), the van der Waals attraction is also greater for deformed vesicles, since there is a contribution from interacting plane parallel bilayers. There are molecular mechanisms for control of the bending constant magnitude,[26] which give an opportunity to control the adhesive behavior as well. Another way to induce or prevent intervesicle adhesion is by varying the repulsive interactions (expressed effectively in our consideration by the distance h). They could be due to electrostatics or steric effects and therefore be sensitive to the ionic strength and temperature of the suspension.

5. Concluding Remarks

This study presents a model which allows one to determine the contribution of nonspecific colloidal forces to the adhesion and aggregation of bilayer lipid vesicles and more specifically the coupling between van der Waals attraction and the bilayer flexibility. The incorporation of other attractive interactions, for example, depletion, is straightforward. Examples for such interactions in the case of emulsion droplets have been published.[14,15,18,19] It is shown that the coupling with the bilayer flexibility may lead to a relatively strong attraction, much stronger than the one provided by the van der Waals interaction alone and in the absence of deformation. The interbilayer repulsion is not considered explicitly but effectively by introducing a minimal distance of possible approach. The origin of the repulsive interactions could be electrostatic (as in the classical DLVO theory[3-8]), steric and hydration,[22] etc. An important contribution to the total interaction between the vesicles is the line excess energy, due to the region surrounding the flattened bilayers. This contribution leads to the appearance of a barrier, which may prevent adhesion for certain experimental conditions. The importance of the bilayer flexibility for the adhesive properties should be present in the case of vesicle substrate interactions. It can explain the observed sensitivity of the vesicle adhesion to glass surfaces upon the lipid phase state.[27]

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