Biomembrane Adhesion to Substrates Topographically Patterned with Nanopits

Jaime Agudo-Canalejo1,2,3,* and Dennis E. Discher4

1Theory & Bio-Systems Department, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany; 2Rudolf Peierls Centre for Theoretical Physics, University of Oxford, Oxford, United Kingdom; 3Department of Chemistry, Pennsylvania State University, University Park, Pennsylvania; and 4Biophysical Engineering, University of Pennsylvania, Philadelphia, Pennsylvania

ABSTRACT We examine the adhesion of biomembranes to substrates topographically patterned with concave nanopits and identify several universal features in the adhesion process. We find three distinct states, depending on whether the membrane remains flat above the nanopit, partially enters it, or completely adheres to it, and derive analytical conditions for the stability of these states valid for a very general class of nanopit shapes. Surprisingly, completely adhered states are always (meta)stable. We also show that the presence of many nanopits can increase or decrease the effective adhesiveness of a substrate, depending on the tension of the membrane and the strength of the membrane-substrate attraction. Our results have implications regarding several experimental methods, which involve the formation of supported lipid bilayers on substrates patterned with nanopits, as well as observations of decreased spreading of cells and migration of cells toward regions of lower nanopit density on topographically patterned substrates. Furthermore, our predictions can also be directly tested in experiments exploring the adhesion of micropipette-aspirated giant vesicles to such substrates.

INTRODUCTION

Thanks to the advances of nanotechnology, it is now possible to fabricate solid substrates of very precise topography, with control over nanoscale features as small as 3 nm in the case of electron beam lithography (1). These topographically patterned substrates are widely used in biophysical research as customizable, synthetic approximations to the nanoscale topography of the extracellular matrix that surrounds living cells (2,3). Topographies commonly investigated include spatial patterns of elongated nanogrooves, convex nanoposts, or concave nanopits on otherwise planar substrates. Variations in the shape and areal density of these features have been shown to strongly affect cell spreading, differentiation, migration, and proliferation (3–11).

Although some theoretical effort has been dedicated to understanding the interaction of biomembranes with rough substrates (12,13) as well as with undulated substrates patterned with nanogrooves (14), the interaction of biomembranes with substrates patterned with nanopits is still not understood. Cells in contact with nanopit-patterned substrates have been shown to display decreased adhesion and spreading in a number of experiments, see (3) for a review. Cells in such substrates have also been observed to migrate toward regions of lower nanopit density (6). Moreover, substrates patterned with nanopits are useful not only in experiments with biological cells but also in more basic biophysical research. In recent years, supported lipid bilayers grown on substrates patterned with concave nanopits (15,16) have been used as platforms for single-molecule spectroscopy of membrane-embedded proteins (17), as well as for the study of membrane curvature sensing by proteins (18).

Given the wide relevance of nanopit-patterned substrates, it is surprising that a basic understanding of the underlying membrane-nanopit interaction is still lacking. Here, we intend to provide a comprehensive theory taking into account both the detailed interactions of the membrane with a single nanopit, as well as the collective effect of many nanopits on membrane adhesion (see Fig. 1). A membrane bound to such a patterned substrate, be it a cell, a giant vesicle, or a supported bilayer, will necessarily be bound to the planar surface of the substrate (see Fig. 1a), but it may or may not adhere to the surface of the nanopits (see Fig. 1b). In fact, three qualitatively different states of the membrane can be distinguished, namely a nonadhered (NA) state if the membrane remains flat above the nanopit, a partially adhered (PA) state if the membrane only shallowly enters the nanopit, and a completely adhered (CA)
state if the membrane is fully bound to the nanopit and closely follows its shape.

We will first examine the stability of these three states for a single nanopit, using both numerical computations, as well as analytical calculations. The analytical calculations show that the general features of the adhesion process are universal, i.e., independent of the detailed shape of the nanopit, which may range from a shallow concavity to a deep cylindrical pit with vertical walls. Then, we will use these results to describe the coarse-grained effect of many nanopits on membrane-substrate adhesion. We will show that the presence of many nanopits endows the substrate with an “effective adhesiveness,” which depends on the tension of the membrane and on the actual adhesive strength of the membrane-substrate attraction. We will relate our results to experiments in the literature concerning cells and supported bilayers. We will focus on symmetric bilayers without spontaneous curvature, and the contributions of Gaussian curvature to the energy can be ignored because we do not consider any changes of the topology of the membrane (19). The second term represents the adhesion energy, which is included via a contact potential where \(|W|\) is the adhesive strength of the membrane-substrate interactions, and \(A_{bo}\) is the area of membrane bound to the substrate. Finally, the third term represents the energetic cost of extracting an amount \(A\) of membrane area from a membrane reservoir at constant tension \(\Sigma\). Biological cells, on the other hand, have internal membrane reservoirs that are actively maintained at a constant tension \(\Sigma\).

Osmotic pressure contributions to the energy in Eq. 1 are negligible if the nanopits are much smaller than the cell or vesicle, and we further assume that there is enough space (a couple of nanometers) between membrane and substrate for the aqueous medium to freely flow in and out of nanopits. We also note that, in the case of cells, interactions with nanopits larger than a few hundred nanometers will necessarily involve rearrangements of the cellular cortex underlying the lipid bilayer. The theoretical results described here are therefore expected to be valid only for nanopit sizes in the range of tens to a couple hundred nanometers in the case of biological cells and giant vesicles but should remain valid for pits of any size in the case of supported bilayers.

### Defining the shape of a nanopit

We rigorously define a smooth axisymmetric nanopit of radius \(R\) by a height function \(z_{np}(r)\), defined on the interval \(0 \leq r \leq R\), that (i) is nondecreasing with only one inflection point in its domain, (ii) is differentiable at least once, and satisfies (iii) \(z_{np}(0) = 0\) and (iv) \(z_{np}(R) = 0\). The first condition ensures that the nanopit has a simple shape with a single minimum at \(r = 0\), whereas the latter three ensure that there are no “kinks” along the nanopit contour, at its bottom, or at the matching line between the nanopit and the planar substrate, respectively. An example for such a height function, which we will use throughout this work, is the polynomial

\[
z_{np}(r) = h\left[3\left(\frac{r}{R}\right)^2 - 2\left(\frac{r}{R}\right)^3\right],
\]

which is plotted in Fig. 1b for \(h/R = 2\).

Besides the general class of nanopit shapes that can be fully described by a height function \(z_{np}(r)\) satisfying the four conditions above, we will also consider the extreme case of cylindrical nanopits with vertical walls. These
constitute a singular kind of nanopit, in that they cannot be completely described by a height function $z_{np}(r)$, because such a height function would be nondifferentiable at the position of the wall $r = R_{wall}$, with $z'_{np}(r = R_{wall}) \to \infty$. As a typical example of such a cylindrical nanopit, we will consider piecewise-defined nanopits such as the one depicted in Fig. 2. Such a nanopit is composed of the following three distinct segments: a toroidal rim of radius $R_{rim}$ that meets smoothly with the planar part of the substrate, a vertical wall corresponding to a cylindrical segment of radius $R_{wall}$ and length $L$, and a bottom segment corresponding to a hemispherical segment of radius $R_{rim}$. In line with the notation above for general nanopits, the radius of the nanopit is then given by $R = R_{wall} + R_{rim}$, and its total height is $h = R_{wall} + L + R_{rim}$.

Throughout this work, we will focus on nanopits with shapes that satisfy the basic conditions of axisymmetry and smoothness just described. The relevance of these conditions will be highlighted in the last subsection of the Discussion, in which we will consider membrane adhesion to nanopits with more general shapes that do not satisfy some of these conditions.

### Energy landscapes and contact curvature condition

Using the standard shooting method for axisymmetric membranes (19), we can numerically calculate the shapes of the unbound membrane segment that minimize the energy in Eq. 1 while smoothly matching the bound segment at any given contact radius $r = r^\ast$. In this way, we can obtain the energy landscapes $E(r^\ast)$ describing the adhesion of the membrane to the nanopit. We say that the NA state is (meta)stable if the energy $E(r^\ast)$ has a boundary minimum at $r^\ast = R$, the CA state is (meta)stable if $E(r^\ast)$ has a boundary minimum at $r^\ast = 0$, and a PA state is (meta)stable if $E(r^\ast)$ has a local minimum at some value $0 < r^\ast < R$. When the energy $E(r^\ast)$ has two minima, this corresponds to the coexistence of two (meta)stable states. In this case, the one with the lowest energy of the two is said to be stable, whereas the other one is said to be metastable. When two (meta)stable states coexist, $E(r^\ast)$ will also have a maximum between these two minima, corresponding to the top of the barrier between the two (meta)stable states. We call this a transition state.

![Diagram of a cylindrical nanopit](image)

**FIGURE 2** Geometry of a cylindrical nanopit, composed of a toroidal rim of radius $R_{rim}$, a vertical wall that corresponds to a cylindrical segment with radius $R_{wall}$ and length $L$, and a hemispherical bottom segment with radius $R_{rim}$. In this particular example, the membrane (yellow) is in a partially adhered (PA) state, with the contact line located at the vertical wall. To see this figure in color, go online.

Alternatively, it is also possible to directly obtain the $r^\ast$-values of all the equilibria present in such an energy landscape (i.e., all the (meta)stable and transition states) without having to calculate the full energy landscape. Indeed, in equilibrium, a subtle balance between stresses and moments at the contact line (20,23) relates the principal curvature of the unbound segment of the membrane perpendicular to the contact line, $C_{\perp}(r^\ast)$, to the curvature of the nanopit at the same line $C_{\perp, np}(r^\ast)$ via $C_{\perp}(r^\ast) = \sqrt{2W/[k + C_{\perp, np}(r^\ast)]}$, or equivalently

$$[W] \equiv k\left[C_{\perp}(r^\ast) - C_{\perp, np}(r^\ast)\right]^2/2.$$

In this case, one can simply obtain $C_{\perp}(r^\ast)$ using the shooting method described above, and then introduce this value into Eq. 3 to obtain the equilibrium values of $r^\ast$, which include both (meta)stable as well as transition states, as a function of the adhesive strength $[W]$. By considering how the equilibrium $r^\ast$-values change with $[W]$ (and knowing that NA and CA states should be energetically preferred at low and high $[W]$), respectively one can obtain the conditions under which NA, PA, or CA states are (meta)stable or become unstable, without the need to examine the energy landscapes explicitly.

### Small gradient approximation

The shape of the unbound membrane $z(r)$ in contact with an axisymmetric nanopit described by a height function $z_{np}(r)$ can be obtained analytically in the small gradient approximation $z'(r) \ll 1$. The general axisymmetric solution is as follows (24):

$$z(r) \approx a_1 + a_2 \log(r/\lambda) + a_3 K_0(r/\lambda) + a_4 I_0(r/\lambda),$$

where $\lambda = \sqrt{\kappa/\Sigma}$, and $I_n(x)$ and $K_n(x)$ are modified Bessel functions of the first and second kind, respectively. The constants $a_i$ are defined by imposing membrane smoothness at $r = 0$ and $r = r^\ast$, leading to

$$z(r) - z(0) \approx [-1 + I_0(r/\lambda)\lambda z_{np}''(r^\ast)]/I_1(r/\lambda),$$

with $0 \leq r \leq r^\ast$ and the small gradient approximation remaining valid whenever $z_{np}''(r^\ast) \ll 1$.

Within this approximation, $C_{\perp}(r^\ast) \approx z'(r^\ast)$ and $C_{\perp, np}(r^\ast) \approx z_{np}''(r^\ast)$. The contact curvature condition in Eq. 3 can then be written explicitly as

$$|W| \approx \frac{k}{2} \left(\frac{I_0(r^\ast/\lambda)}{\lambda I_1(r^\ast/\lambda)} z_{np}(r^\ast) - z_{np}''(r^\ast)\right)^2.$$  

### Curvature of a membrane in contact with a vertical wall

In the particular case of cylindrical nanopits with a vertical wall, it is possible to calculate exactly the curvature $C_{\perp}(r^\ast = R_{wall})$ of the membrane at the contact line where the membrane meets the wall. Indeed, suppose that the membrane is in an equilibrium state in which the contact line is located at the vertical wall (see Fig. 2). Because the shape of the unbound segment of the membrane does not depend on the height of the contact line along this wall, i.e., on how deep into the nanopit the membrane is, the energetic cost of moving this contact line up or down will be determined only by the energy of the bound segment. The energetic cost of moving the contact line downward a distance $\delta z$ is therefore

$$\delta E = \left(\frac{2k}{(2R_{wall})}\right) - [W] + \Sigma 2\pi R_{wall} \delta z.$$
This energetic cost will be zero, and thus the membrane will be in equilibrium, if the adhesion contribution to the energy can compensate for both the bending and tension contributions, with

$$|W| = \Sigma + \frac{\kappa}{2R_{\text{wall}}^2}. \quad (8)$$

Eq. 8 represents an equilibrium condition valid for the particular case in which the substrate-membrane contact line is at a vertical wall. However, in such a situation, the membrane must also satisfy the general equilibrium condition in Eq. 3. Putting both equations together and noting that at the vertical wall we have $C_{\perp}(r^* = R_{\text{wall}}) = 0$, we conclude that the curvature of the membrane at the contact line with the wall is

$$C_{\perp}(r^* = R_{\text{wall}}) = \frac{2\Sigma}{\kappa} + \frac{1}{R_{\text{wall}}^2}. \quad (9)$$

RESULTS

Membrane interaction with a single nanopit

Let us first consider the interaction of the membrane with a single nanopit. In Fig. 3a, we plot the numerically calculated equilibrium contact radius $r^*$ of the membrane as a function of the adhesive strength $|W|$ for an example nanopit with shape given by Eq. 2 with $h/R = 0.1$, for three representative values of membrane tension $\Sigma$. The curves show that CA states are always (meta)stable, for all values of $|W|$ and $\Sigma$, and that the transition from NA or PA to CA states is always discontinuous. For low tension, we find that increasing $|W|$ leads to an instability $L_{\text{na}}$ of NA states toward CA states, whereas for tensions above a critical value $\Sigma = 4.7\kappa/R^2$, NA states first transition continuously toward PA states, which then undergo an instability $L_{\text{pa}}$ toward CA states for even higher values of $|W|$. The value of $|W|$ corresponding to the instability $L_{\text{na}}$ is found to be independent of membrane tension and is given by $|W| = 0.18\kappa/R^2$. At the discontinuous transition $D$, marked by vertical lines, the NA or PA states have equal energy as the CA states. These results are summarized in the corresponding stability diagram in Fig. 3b, describing the stability of NA, PA, and CA states as a function of $|W|$ and $\Sigma$. The features just described can also be seen in the energy landscapes that we display in Fig. 4, corresponding to the case of a membrane with tension $\Sigma = 30\kappa/R^2$ and eight different values of the adhesive strength $|W|$, which correspond to the eight crosses in Fig. 3b. In particular, one can directly see in the energy landscapes that the CA state is (meta)stable for all values of $|W|$, as well as how the discontinuous transition $D$ and the instability lines of the NA and PA states are crossed with increasing adhesive strength.

The small gradient approximation in Eq. 6 is plotted as the black long-dashed lines in Fig. 3a. This approximation works very well for shallow nanopits such as the one in Fig. 3a but becomes increasingly inaccurate for deeper nanopits with higher $h/R$ and ultimately breaks down for nanopits with vertical walls (see next subsection). Importantly, however, the approximation in Eq. 6 can make exact predictions regarding the stability limits of the CA state ($r^* = 0$) and the NA state ($r^* = R$), because in these two limits the unbound membrane segment becomes exactly flat with $z'(r) \equiv 0$, independently of the nanopit depth.

In fact, we will now use Eq. 6 to show that the defining features of the membrane-nanopit interaction in Fig. 3 are universal, i.e., independent of the specific shape of the
FIGURE 4  Energy landscapes of adhesion $\Delta E(r^*) = E(r^*) - E(R)$ for the nanopit in Fig. 3 and for membrane tension $\Sigma R^2/k = 30$ and eight different values of the adhesive strength $|W|$, corresponding to the eight crosses in the stability diagram of Fig. 3. It can be directly seen that, for all values of $|W|$, the CA state at $r^* = 0$ is (meta)stable. With increasing $|W|$, the system crosses first the instability line $I_{\text{pa}}$, then the discontinuous transition $D$ where the PA and CA states switch metastability, and finally the instability line $I_{\text{np}}$ of the PA state toward the CA state. To see this figure in color, go online.

meets the planar substrate with $C_{\text{rim}} = 0$, i.e., without a curvature discontinuity, the NA state is unstable even for vanishing adhesion. For the example in Fig. 3, Eq. 12 with $z_{np}''(R) = -6h/R^2$ predicts $|W| = 18kh^2/R^4$, resulting in $|W|/R^2/k = 0.18$ for $hR = 0.1$, which coincides with the numerical result in Fig. 3. We note that Eq. 12 could have been directly obtained from the contact curvature condition in Eq. 3, without recurring to the small gradient approximation, by noticing that in the NA state we have $C_\perp(R) = 0$ and $C_{\perp np}(R) = C_{\text{rim}}$.

Third, using Eq. 6, we can find a condition for the existence of a (meta)stable PA state by imposing that $d|W|/dr^*|_{r^* = R} < 0$. The condition reads

$$\frac{1}{\lambda} \left( \frac{1}{\lambda} I_0(R/\lambda) + I_2(R/\lambda) \right) - \frac{R}{\lambda} z_{np}(R) - R z_{np}''(R) < 0,$$

and, taking into account that $z_{np}''(R)$ is always negative and that $x[I_0(x) + I_2(x)]/I_1(x)$ is a monotonically increasing function for $x > 0$, it defines a critical value of $R/\lambda$, and therefore also defines a critical value of the membrane tension through $1/\lambda \equiv \sqrt{\Sigma/k}$, above which (meta)stable PA states exist. For the example in Fig. 3, with $z_{np}''(R) = -12h/R^3$, we find $R/\lambda \approx 2.17$, which means that the triple point at which the $L_{\text{na}}$ and $L_{\text{np}}$ lines meet is located at $\Sigma R^2/k \approx 2.17^2 \approx 4.7$, coinciding with the numerical result in Fig. 3. Remarkably, the condition in Eq. 13 is invariant under the vertical scaling transformation $z_{np}(r) \rightarrow \alpha z_{np}(r)$, which implies that the critical tension remains the same if the shape of a nanopit is vertically “stretched” ($\alpha > 1$) or “compressed” ($0 < \alpha < 1$). For example, both the deep nanopit in Fig. 1 b and the shallow nanopit in the inset of Fig. 3 a have the same critical tension given by $\Sigma R^2/k = 4.7$. This is because both are described by the same height function in Eq. 2, the former with $hR = 2$, the latter with $hR = 0.1$, and therefore both are related by a vertical scaling transformation. Furthermore, by evaluating Eq. 13 at $R/\lambda = 0$, we find the condition

$$z_{np}''(R) - R z_{np}'''(R) < 0,$$

for PA states to exist for all values of $\Sigma \geq 0$; that is, nanopits that satisfy Eq. 14 will not display a triple point in their stability diagram.

Finally, let us examine the limit of large membrane tension, with $\Sigma R^2/k \gg 1$. In this limit, bending is negligible and the membrane will behave as a flat liquid interface satisfying the Young-like equation $|W| \approx \Sigma (1 - \cos \theta)$, where $\theta = \arctan z_{np}'(r^*)$ is the angle between the substrate and the horizontal plane at the contact line $r^*$. First, notice that the function $1 - \cos(\arctan \theta)$ is monotonically increasing for $x > 0$. Second, the function $z_{np}'(r^*)$ will have a maximum at the value $r^* = r_{\text{in}}$ at which the height function $z_{np}'(r^*)$ has an inflection point (i.e., when $z_{np}''(r^*) = 0$). The PA state will become unstable.
toward the CA state for adhesive strengths larger than the maximum of $|W|$ as a function of $r^*$, which is given by

$$|W| \approx \Sigma \left\{ 1 - \cos \left[ \arctan z_{np}(r_{in}) \right] \right\}, \quad (15)$$

and represents the large tension behavior of the stability limit $L_{pa}$. For the nanopit in Fig. 3, we find $r_{in} = R/2$ and $z_{np}(r_{in}) = (3/2)(h/R)$, leading to $|W| \approx 0.011 \Sigma$ which is plotted as the red dotted line in Fig. 3b.

The interaction of a membrane with a nanopit of arbitrary shape (as long as this shape is axisymmetric and smooth, satisfying the conditions introduced in Defining the Shape of the Nanopit) can therefore be universally described in the following way. The CA state will be always at least metastable for all values of the adhesive strength $|W|$ or the membrane tension $\Sigma$. For low values of the adhesive strength $|W|$, lower than the tension-independent threshold given by Eq. 12, this CA state will coexist with the NA state. For values of $|W|$ larger than this threshold, the NA state will typically become unstable toward a PA state, but it may also become unstable directly toward the CA state for certain nanopits if the membrane tension is low enough (see Eqs. 13 and 14). Even for large tensions, however, the PA state ultimately becomes unstable toward the CA state for large enough adhesive strengths, as approximated by Eq. 15 in the limit of large membrane tension.

**Membrane interaction with a single cylindrical nanopit**

An important class of nanopits corresponds to cylindrical nanopits with vertical walls (see Fig. 2). Cylindrical nanopits covered by a supported lipid bilayer have been used, for example, as waveguides for single-molecule spectroscopy of membrane-embedded proteins (17). Such nanopits are particularly easy to produce and simple to describe, because their geometry is fully determined by their radius, their depth, and the curvature of their rim. Cylindrical nanopits with vertical walls represent an extreme, singular example of the general smooth axisymmetric nanopits that we have described above. One may thus wonder how the general results described above apply to cylindrical nanopits. In Fig. 5a, we plot the numerically calculated equilibrium contact radius $r^*$ of the membrane as a function of the adhesive strength $|W|$, for a cylindrical nanopit with cylinder radius $R_{wall} = 0.8R$, rim radius $R_{rim} = 0.2R$, and arbitrary wall length $L$, for a representative value of the membrane tension $\Sigma$. The corresponding stability diagram representing the stability of NA, PA, and CA states as a function of $|W|$ and $\Sigma$ is depicted in Fig. 5b. The discontinuous transition $D$ at which NA or PA states switch metastability with CA states is not displayed in this case, because it does depend on the specific choice of wall length $L$.

**FIGURE 5** Interaction with a single cylindrical nanopit: (a) the equilibrium radius of the contact line $r^*$ as a function of adhesive strength $|W|$, for tension $2\kappa R^2/\kappa = 10$ which is representative of all other values of the tension $\Sigma$; and (b) a stability diagram displaying the coexistence of nonadhered (NA), partially adhered (PA), and completely adhered (CA) states as a function of $|W|$ and $\Sigma$. The shape of the nanopit is determined by $R_{wall} = 0.8R$, $R_{rim} = 0.2R$, and arbitrary $L$. In contrast to the latter figure, we do not indicate the location of the discontinuous transition $D$, which would depend on the specific value of the wall height $L$. The meaning of the labels i, ii, iii, “hs-wall,” and “wall-rim” in (a) is explained in the text. To see this figure in color, go online.

As expected, the small gradient approximation in Eq. 6, which corresponds to the black long-dashed line in Fig. 5a, completely breaks down in the proximity of the vertical wall when $r^* = R_{wall}$. Nevertheless, it is still accurate in describing the stability of the CA and NA state, at $r^* = 0$ and $r^* = R$, respectively. Indeed, we first notice that the CA state is again (meta)stable for all values of the adhesive strength $|W|$ or the membrane tension $\Sigma$, as predicted by Eq. 11 above. Secondly, the NA state again becomes unstable for sufficiently large values of the adhesive strength $|W|$, beyond the tension-independent stability line $L_{na}$. As predicted by Eq. 12, $z_{np}(R) = C_{rim} = -1/R_{rim}$, the stability line $L_{na}$ is in this case given by...
The values of $|W|$ obtained from curvatures (ii) and (iii) also have a physical meaning. For adhesive strengths in the interval obtained from curvatures (i) and (ii)

$$\frac{\kappa}{2} \left[ \sqrt{\frac{2\Sigma}{\kappa} + \frac{1}{R_{\text{wall}}} + \frac{1}{R_{\text{rim}}}} \right]^2 > |W| > \Sigma + \frac{\kappa}{2R_{\text{wall}}^2},$$

the transition state corresponding to the top of the energy barrier separating the NA or PA state from the CA state is represented by the state in which the contact line is located at the line where the rim and the vertical wall meet. This range of values of $|W|$ is indicated by the label “wall-rim” in Fig. 5a. For adhesive strengths with $|W| = \Sigma + \kappa/2R_{\text{wall}}$, corresponding to point (ii), the transition state is degenerate, and the top of the energy barrier is a “plateau” corresponding to the contact line being located anywhere along the wall of the nanopit. For adhesive strengths in the interval obtained from curvatures (ii) and (iii)

$$\Sigma + \frac{\kappa}{2R_{\text{wall}}^2} > |W| > \frac{\kappa}{2} \left[ \sqrt{\frac{2\Sigma}{\kappa} + \frac{1}{R_{\text{wall}}} + \frac{1}{R_{\text{rim}}}} \right]^2,$$

the transition state is represented by the state in which the contact line is located at the line where the hemispherical bottom segment and the vertical wall meet. This range of values of $|W|$ is indicated by the label “hs-wall” in Fig. 5a. Finally, for even lower adhesive strengths, the transition state will be represented by states where the contact line is somewhere along the hemispherical bottom segment.

In summary, the interaction of membranes with cylindrical nanopits of arbitrary radius, rim curvature, or depth can be described in the following way. As in the case of noncylindrical nanopits, the CA state will be always at least metastable for all values of the adhesive strength $|W|$ or the membrane tension $\Sigma$. For low values of the adhesive strength $|W|$, below the tension-independent threshold given by Eq. 16, this CA state will coexist with the NA state. Surprisingly, this threshold value is independent of the nanopit radius or depth and only depends on the radius of curvature of the rim. For values of $|W|$ larger than this threshold, the NA state becomes unstable toward a PA state, independently of the membrane tension. Finally, for adhesive strengths larger than the threshold given by Eq. 17, the PA state becomes unstable toward the CA state. This latter threshold is dependent on the tension of the membrane but again does not depend on the depth of the nanopit. Moreover, these two important thresholds are independent of the shape of the bottom of the nanopit. The main results described here are therefore valid even if the bottom of the cylindrical nanopit has a shape other than hemispherical.
Effective adhesiveness of substrates patterned with many nanopits

Now that we understand the interaction of the membrane with single nanopits, we turn to the collective effect of many nanopits on membrane-substrate adhesion (see Fig. 1 a). Suppose that a membrane is in contact with the substrate over a “projected” area \( A_{xy} \) on the horizontal plane and that this projected area spans \( N \) nanopits. The total energy of the system is given by \( E = (\Sigma - |W|)(A_{xy} - N\pi R^2) + NE_{np,i} \), where \( E_{np,i} \) is the interaction energy with a single nanopit. The subindex \( i \) can take the values \( i = \text{NA}, \text{PA}, \text{CA} \) corresponding to the three possible states of the membrane-nanopit complex, depending on which of these states are (meta)stable. We can now define the effective adhesiveness \( |W|_{\text{eff}} \) of an equivalent, coarse-grained planar substrate without nanopits by rewriting the energy of the system as \( E = (\Sigma - |W|_{\text{eff}})A_{xy} \). Equating both expressions, we obtain

\[
|W|_{\text{eff}} = |W| + \Gamma(\Sigma - |W| - E_{np,i}/\pi R^2),
\]

where \( \Gamma = N\pi R^2/A_{xy} \) is the fraction of the projected area of the substrate that is covered in nanopits, which can range from \( \Gamma = 0 \) in the absence of nanopits to a maximum of \( \Gamma = \pi/2\sqrt{3} \approx 0.9 \) for an optimal hexagonal packing of nanopits.

This effective adhesiveness, rescaled as \( (|W|_{\text{eff}}/|W| - 1)/\Gamma \), is plotted in Fig. 6 a for a substrate patterned with nanopits such as the one in Fig. 3 as a function of membrane tension for the three values of real adhesive strength \( |W| \) indicated by the arrows in Fig. 3 b. In general, \( |W|_{\text{eff}} \) decreases with increasing membrane tension. Because the membrane interactions with a single nanopit showed bistable behavior, with NA and CA or PA and CA states sometimes coexisting, \( |W|_{\text{eff}} \) can also show two coexisting branches. Solid lines correspond to every nanopit being in its lowest-energy stable state, which in turn translates into higher values of \( |W|_{\text{eff}} \). The lower values of \( |W|_{\text{eff}} \), corresponding to all nanopits staying in metastable NA, PA, or CA states, are plotted as dashed, dashed-dotted, and dotted lines, respectively.

The existence of bistable behavior should manifest itself as hysteresis in the effective adhesiveness when the membrane tension is increased or decreased. As an example, consider the case with \( |W| R^2/\kappa = 0.25 \) depicted in Fig. 6 a. Adhesion measurements at high tension, to the right of \( L_{pa} \), are expected to explore the PA branch. If, however, once in contact with the substrate the tension of the membrane is sufficiently decreased (to the left of \( L_{pa} \)), the PA branch will become unstable, and the system will fall into the CA branch with higher effective adhesiveness. Even if the tension is now increased back to its original value, the system will typically remain in the metastable CA branch, with effective adhesiveness lower than at the beginning of the cycle. The hysteretic cycle just described corresponds to the points 1–4 indicated in Fig. 6 a.

For NA states, the interaction energy with a single nanopit is simply \( E_{np,\text{NA}} = 2\pi R^2 \), and Eq. 20 naturally predicts a decreased adhesiveness in the presence of nanopits, with \( |W|_{\text{eff}} = |W|(1 - \Gamma) < |W| \) or equivalently \( (|W|_{\text{eff}}/|W| - 1)/\Gamma = -1 \). For PA states, we also find decreased adhesiveness, slightly larger but close to that of NA states, so that \( (|W|_{\text{eff}}/|W| - 1)/\Gamma \geq -1 \). For CA states, on the other hand, the rescaled \( |W|_{\text{eff}} \) depends

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**FIGURE 6** (a) Rescaled effective adhesiveness \( |W|_{\text{eff}} \) for a substrate patterned with nanopits such as the one in Fig. 3 a, with \( h/R = 0.1 \), as a function of tension \( \Sigma \), for three different values of the real adhesive strength \( |W| \), corresponding to the three arrows in Fig. 3 b. Solid lines correspond to all nanopits being in the lowest energy state, which might be NA, PA, or CA; whereas dashed, dashed-dotted, and dotted lines correspond to metastable NA, PA, and CA states, respectively. The points 1–4 represent a hysteresis cycle of decreasing/increasing tension, as described in the text. (b) Rescaled \( |W|_{\text{eff}} \) at \( \Sigma = 0 \) as a function of \( |W| \), for the same type of nanopits as in (a) but for three different values of \( h/R \). The corresponding nanopit shapes are depicted in the inset. Horizontal dashed lines indicate the asymptotes for large \( |W| \) given by Eq. 23. To see this figure in color, go online.
strongly on both $|W|$ and $\Sigma$. Indeed, the interaction energy for a CA state can be written as $E_{np,CA} = (\Sigma - |W|)A_{np} + E_{be, np}$, where $A_{np} > \pi R^2$ is the surface area of the nanopit, and $E_{be, np} = 2k_{ij}A_{np}M^2dA > 0$ is the bending energy of the membrane adhering to it. Introducing $E_{np,CA}$ into Eq. 20, we find
\[
\left(\frac{|W|_{\text{eff}}}{|W|} - 1\right) \frac{1}{\Gamma} = \left(\frac{|W| - \Sigma}{|W|} \right) \left(\frac{A_{np} - \pi R^2}{|W|} - E_{be, np}\right),
\]
which increases both with increasing $|W|$ and with decreasing $\Sigma$. In fact, Eq. 21 predicts an increased adhesiveness in the presence of nanopits, with $|W|_{\text{eff}} > |W|$, whenever the numerator is positive, i.e., when
\[
|W| - \Sigma > \frac{E_{be, np}}{A_{np} - \pi R^2},
\]
and a decreased adhesiveness otherwise. Furthermore, in the limit of very adhesive substrates with $|W|$ much larger than both $\Sigma$ and $k/R^2$, Eq. 21 predicts that the nanopit-induced increase in substrate adhesiveness saturates to
\[
\left(\frac{|W|_{\text{eff}}}{|W|} - 1\right) \frac{1}{\Gamma} \approx \frac{A_{np}}{\pi R^2} - 1,
\]
which becomes larger with increasing nanopit depth.

To illustrate this behavior, we have plotted in Fig. 6b the rescaled $|W|_{\text{eff}}$ as a function of $|W|$, for $\Sigma = 0$ and three different values of $h/\rho$, i.e., of nanopit depth. We focus only on the stable branches, which are the NA branch for low adhesion and the CA branch for higher adhesion, beyond the discontinuous transition $D$. The asymptotes for large $|W|$ given by Eq. 23 are represented by the horizontal dashed lines. As predicted, for low $|W|$, the presence of nanopits acts to decrease the effective adhesiveness of the substrate, with $|W|_{\text{eff}} < |W|$, whereas for sufficiently high $|W|$ the nanopits increase surface adhesiveness, with $|W|_{\text{eff}} > |W|$. Furthermore, for low values of $|W|$, the effective adhesiveness decreases both with increasing nanopit depth as well as with increasing nanopit coverage $\Gamma$; the opposite is true for high values of $|W|$.

**DISCUSSION**

**Supported lipid bilayers**

Supported lipid bilayers grown on substrates topographically patterned with nanopits have recently been proposed as platforms for single-molecule spectroscopy of membrane-embedded proteins (17), as well as for the study of membrane curvature sensing by proteins (18). In these experimental methods, it is essential that the supported bilayer closely follows the shape of the nanopits, i.e., be found in the CA state. It is therefore important to understand under which conditions we can expect to find CA states over NA or PA states.

The tension of a supported membrane is fixed by the adhesiveness of the substrate itself, with $\Sigma = |W|$ (25). This can be intuitively understood by realizing that, because the total membrane area of the supported bilayer is fixed, any new area $\delta A$ brought into the nanopit was previously adhering to the flat part of the substrate, which implies an energetic cost $\delta E = |W|\delta A$. In the case of supported bilayers, therefore, the adhesive strength and the membrane tension are not independent variables, and we can only explore one-dimensional cuts $\Sigma = |W|$ through the two-dimensional $(|W|, \Sigma)$ stability diagrams such as those in Figs. 3b and 5b.

Supported lipid bilayers are typically grown by the adhesion and rupture of vesicles onto the substrate (15,16). Our results imply that how a supported bilayer in a CA state can be successfully generated will depend on the size of these vesicles, in particular, on whether the vesicles are smaller or larger than the size of the nanopit. First, let us consider the case of vesicles that are smaller than the nanopit. Rupture of the vesicles onto the surface of the nanopit will generate a CA state from the beginning, and the important question is then whether this initial CA state will remain stable. Luckily, our results above show that CA states are (meta)stable for all values of the adhesive strength $|W|$ and independently of the nanopit shape. It is therefore expected that supported bilayers in a CA state, i.e., closely following the shape of the nanopits, can be easily generated from the adhesion and rupture of sufficiently small vesicles onto the substrate, in the sense that the vesicles are small enough to enter the nanopit. Theoretical studies show that small vesicles can adhere to concave surfaces more easily than to flat surfaces (26,27).

Now, let us consider the case of supported membranes generated by the adhesion and rupture of vesicles that are larger than the nanopits, in the sense that they are too large to enter the nanopit. Rupture of such vesicles onto the substrate will lead to the initial formation of NA or PA states, and the important question is then whether these states will be unstable toward a CA state. Here, we find an interesting behavior. Inspection of Fig. 3b, corresponding to a rather shallow and flat nanopit, shows that the line $\Sigma = |W|$ will cross the instability line $L_{na}$ at $|W| = 0.18\kappa/R^2$ and remain below the instability line $L_{pa}$ for all larger $|W|$-values. Therefore, any sufficiently large adhesive strength will spontaneously lead to a CA state for a supported membrane in contact with such nanopits. On the other hand, inspection of Fig. 5b, corresponding to a cylindrical nanopit with vertical walls, shows that the line $\Sigma = |W|$ will cross the instability line $L_{na}$ but not the instability line $L_{pa}$, at least within the area of the figure. This implies that supported membranes in contact with such nanopits will remain in (meta)stable PA states even for very adhesive substrates with large $|W|$.
The transition between these two regimes can be understood from the limiting behavior of the instability line $L_{pa}$ at large membrane tension, given by Eq. 15. Indeed, this equation can be rewritten as $\Sigma \approx \left\{ \frac{1 - \cos \left[ \arctan z_{np} \left( \frac{r_m}{r_p} \right) \right]}{c_{np}} \right\}^{-1} |W| \equiv \alpha |W|$. As long as the nanopit does not have vertical walls, we find $\alpha > 1$, and the line $\Sigma = |W|$ will eventually cross the instability line $L_{pa}$. As the walls of the nanopit are made more vertical, the value of $\alpha$ becomes closer to one, and the two lines become more parallel to each other, leading to an increase in the value of $|W|$ at which the two lines cross, and the PA state becomes unstable for a supported membrane. Ultimately, for a nanopit with vertical walls, we find $\alpha = 1$, implying that the two lines become exactly parallel and do not cross for any $|W|$-value. Therefore, in the extreme case of nanopits with vertical walls (and only in this case), PA states will remain metastable for all values of the adhesive strength $|W|$. This can be seen explicitly by comparing $\Sigma = |W|$ with the exact expression of $L_{pa}$ for cylindrical nanopits (see Eq. 17). For all other nanopits with nonvertical walls, there will be a value of $|W|$ above which both NA and PA states are unstable toward CA states.

After the vesicles (large or small) adhere and rupture onto the substrate, the different patches of membrane arising from this rupture process will spread and creep across the surface and stitch together into a uniform and continuous supported bilayer (28). In the presence of nanopits, the energetics and dynamics of this “coarsening” process will be rather subtle and should depend on the relative size of the membrane patches 1) with respect to the size of the nanopits, as well as 2) with respect to the typical distance between nanopits. As an example of the former, note that whereas a small membrane patch may “sense” the local curvature of a nanopit larger than itself (small displacements of the patch will affect both its bending energy as well as the length of its edge and therefore its edge energy (29)), a large membrane patch that covers a whole nanopit will be trapped in a degenerate metastable state. Small displacements of the patch will not affect its energy as long as the nanopit is fully contained within the boundaries of the patch. As an example of the latter, note that a membrane patch that is smaller than the typical distance between nanopits may simply “avoid” entering a nanopit if this is energetically unfavorable (we note, however, that entering a nanopit may also be energetically favorable; whereas the bending energy of the patch will necessarily increase, its edge energy may decrease if the length of the patch boundary is reduced in the process). A membrane patch that is larger than the typical distance between nanopits, however, may only “avoid” a nanopit by deforming the shape of its boundary. If the energetic cost of this process is too large, it will be favorable for the membrane patch to instead spread over the nanopit, leading either to an NA, PA, or CA state depending on the adhesive strength of the substrate. All in all, we expect the coarsening process of supported lipid bilayers on substrates with complex topography to show a rich spectrum of behaviors.

**Giant unilamellar vesicles**

A different model system that can be used to experimentally test the theoretical results described here is provided by giant unilamellar vesicles (GUVs). In the context of testing our predictions, it is important to note that an isolated GUV has a fixed number of lipids on its membrane and therefore a certain optimal area. The mechanical tension of its membrane is coupled to the lateral stretching or compression of the membrane above or below this optimal area. Therefore, for an isolated GUV, tension is not a fixed parameter and instead depends in a complex manner on the shape and geometric constraints of the vesicle, as well as on the adhesiveness of the substrate (30).

Alternatively, one may perform experiments using micropipette-held vesicles. In a micropipette setup, part of the GUV is aspirated (the “tongue”), whereas the majority of the GUV remains outside the micropipette. By controlling the aspiration pressure, and therefore the length of the tongue, the tension of the membrane becomes an externally tunable control parameter. With respect to small perturbations of the part of the GUV outside the micropipette, the membrane area stored in the tongue then behaves as a membrane reservoir at a fixed tension $\Sigma$ (31).

Such a micropipette setup is ideal to explore the stability of NA, PA, and CA states as described here (see Figs. 3 b and 5 b), because $|W|$ and $\Sigma$ can be varied independently from each other. However, directly determining the specific state of a membrane in contact with a single nanopit may prove experimentally challenging. In this case, one could directly explore the effective adhesiveness $|W|_{\text{eff}}$ of a substrate patterned with many such nanopits. Indeed, suppose that a micropipette-held GUV is pressed against a topographically patterned substrate in such a way that the vesicle-substrate contact area extends over a large number of nanopits. Measurements of the force required to detach the GUV from the substrate can then be used to gauge the effective adhesiveness $|W|_{\text{eff}}$ of the substrate (31–34).

Of particular interest will be to explore the effective adhesiveness of the substrate as a function of the tension of the membrane. As shown in Fig. 6 a, the effective adhesiveness is generally expected to decrease with increasing membrane tension. Moreover, due to the bistable coexistence of NA and CA or PA and CA branches, the system should exhibit hysteresis behavior when subject to cycles of increasing and decreasing tension, as described above. One example of a hysteresis cycle of decreasing and increasing tension is depicted by the points 1–4 in Fig. 6 a. In the case of a micropipette-held GUV, this would correspond to decreasing and increasing the aspiration pressure.
We note that, in this work, we have focused on the adhesion of vesicles to nanopits (i.e., pits that are much smaller than the vesicle) so that the vesicle-substrate contact region is expected to cover many such pits. The opposite situation, corresponding to a vesicle adhering to a concave pit of size comparable to or larger than the size of the vesicle, has also been studied theoretically (26,27). In the latter works, it was shown that the critical adhesive strength necessary for binding of the vesicle to the substrate is lower in concave pits than in flat substrates.

Biological cells

The adhesion of biological cells to substrates topographically patterned with nanopits has been explored in a number of experiments (3–10,15–18). A common observation in the majority of these experiments has been a decrease in cell spreading in the presence of nanopits with respect to the same substrate in the absence of nanopits (see (3) for a review). Another particularly interesting observation, in (6), is that cells tend to migrate toward regions of lower nanopit density.

Both types of observations seem to suggest that in these experiments the presence of nanopits caused a reduction of the effective adhesiveness of the substrate, with \(|W|_{\text{eff}} < |W|\). Our findings imply (see Eq. 22 and Fig. 6 b) that these trends may be reversed in more sticky substrates with larger adhesive strength \(|W|\), as well as for cells with lower membrane tension \(\Sigma\). Alternatively, it may be possible to engineer the shape of the nanopits to make the right-hand side of Eq. 22 as small as possible, so as to minimize the critical value of the adhesive strength above which nanopits begin to enhance the effective adhesiveness of the substrate. In regard to this we note that, as can be inferred from Fig. 6 b, this critical value of \(|W|\) is only very weakly dependent on the depth of the nanopit (i.e., on the value of \(h/R\)) and instead depends more strongly on the finer details of the nanopit shape.

Membrane rupture and defect formation

Throughout the study, we have assumed at all times that the membrane will remain continuous and intact in the presence of the nanopits, i.e., that the membrane will not rupture or form defects to avoid regions of high curvature. Is this assumption justified? As an example, let us consider a nanopit with a strongly curved rim with radius \(R_{\text{rim}} \ll R\) such as the one in Fig. 2. The energetic cost for the membrane to be bound to this rim can be approximated as a quarter of the bending energy of a cylinder with radius \(R_{\text{rim}}\) and length \(2\pi R\), giving \(E_{\text{be rim}} \approx (\pi^2/2)\kappa (R/r_{\text{rim}})\). On the other hand, if the membrane ruptured to avoid adhering to said rim, this process would require the formation of two membrane edges of length close to \(2\pi R\), with an energetic cost \(E_{\text{edg}} \approx \lambda_{\text{edg}} 4\pi R\), where \(\lambda_{\text{edg}}\) is an edge tension or edge energy per unit area (29). The typical value for such an edge tension, found in experiments and simulations, is about \(\lambda_{\text{edg}} \approx 10\ \text{pN} = 10^{-20}\ \text{J/nm}\) (35). Rupture of the membrane to avoid the highly curved rim will be energetically favored whenever \(E_{\text{edg}} < E_{\text{be rim}}\), which results in the condition \(r_{\text{rim}} < (\pi/8)(\kappa/\lambda_{\text{edg}})\), implying that, indeed, the membrane prefers to rupture at very strongly curved regions. However, introducing the typical values for \(\lambda_{\text{edg}}\) and the bending rigidity \(\kappa \approx 10^{-19}\ \text{J}\), we find \(r_{\text{rim}} < 3.9\ \text{nm}\). As a consequence, we expect that rupture of the membrane will only be preferred at extremely sharp “kinks” of the substrate, with curvature radii comparable to the thickness of the membrane itself.

Multicomponent membranes

Above, we have focused on laterally homogeneous membranes with a symmetric bilayer, i.e., on membranes that can be described as having the same bending rigidity \(\kappa\) and zero spontaneous curvature \(m = 0\) everywhere. Strictly, such a description should be expected to apply only to membranes consisting of a single lipid species or a mixture of very similar lipids. In general, however, biomembranes may be composed of many different lipids and membrane proteins. These individual components may have different affinities toward the substrate or different preferred curvatures, leading to adhesion- or curvature-induced enrichment or depletion of certain components in specific regions. The compositions of the two monolayers that make the bilayer may vary independently, leading to a spatially inhomogeneous spontaneous curvature of the membrane (36). Moreover, the different components can genuinely phase-separate, forming well-defined domains of one phase in a surrounding matrix of the other phase, with each phase having different curvature-elastic and adhesive properties. Indeed, model membranes with as little as three components can phase-separate into two macroscopic fluid phases, a liquid-ordered (Lo) and a liquid-disordered phase (37). What effect will this added complexity have on the adhesion of biomembranes to substrates patterned with nanopits?

CA states will still be always (meta)stable, given that this behavior is independent of the material parameters of the membrane and therefore independent of the details of the local membrane composition at the bottom of the nanopit. The stability limits of NA and PA states, however, will depend on how the membrane components self-organize around the nanopit and thus on the details of the membrane composition. The basic qualitative result indicating that, for weak adhesion, NA or PA states are energetically preferred, whereas, for sufficiently strong adhesion, CA states are preferred, will remain valid independently of the compositional complexity of the membrane. However, the specific values of adhesion at which each state becomes energetically preferred may shift due to a multitude of effects. For example, if some of the membrane components have nonzero preferred curvatures, the cost of bending the
membrane to adapt to the nanopit shape will be lowered, and CA states will be favored by enrichment of these components near the nanopit. Similarly, CA states may be favored by the existence of phase-separated domains, because adhesion of the domains to the nanopits will reduce the length of the domain boundary and therefore the line tension energy of the system. On the other hand, if a membrane were composed of a mixture of lipids, some of which are attracted to the substrate whereas others are not (or are repelled from the substrate), we would expect NA states to be favored, because, in this way, the latter components may avoid the substrate by accumulating in the NA regions of the membrane above the nanopits. One may think of many other factors that will similarly favor one state over the other two. Because of the high dimensionality of the parameter space, developing a general theory of adhesion of multicomponent membranes to nanopit-patterned substrates is a difficult task.

Ignoring the transitions between states, however, one may ask how the presence of the nanopits will affect the lateral organization of the membrane components. Membranes in a CA state and membranes in an NA state will behave rather differently in this regard. In a CA state, the whole membrane is adjacent to the substrate, but the curvature of the membrane is nonuniform. In this case, the different membrane components may reorganize to adapt to this nonuniform curvature. Experiments with lipid bilayers made from ternary mixtures supported on undulated substrates with weakly curved plateaus and strongly curved grooves have shown that the more rigid Lo domains will localize at the plateaus and avoid the grooves (38). Theoretical modeling of the same system (39) shows that localization of Lo domains will occur if the length scale given by \((\kappa_o - \kappa_d)/\lambda\) exceeds a certain critical value related to the topographical features of the system (here, \(\kappa_o\) and \(\kappa_d\) are the bending rigidities of the Lo and liquid-disordered phase, and \(\lambda\) is the line tension of the domain boundary). Otherwise, the membrane will “ignore” the topography of the substrate and simply phase-separate into two large domains. This implies that localization will occur only if the rigidity contrast between the two phases is sufficiently large or the line tension is sufficiently small. A similar result may be expected to hold for a nanopit-patterned substrate, with rigid Lo domains avoiding the nanopits and localizing at the flat regions between them.

In an NA state, on the other hand, the whole membrane is flat, but now some regions of the membrane are adjacent to the substrate whereas others are not. This will lead to a reorganization of the membrane components depending on their affinity toward the substrate, with high-affinity components being enriched in the adhering region outside the nanopits and low-affinity components being enriched in the nonadhering regions above the nanopits. Once again focusing on phase-separating mixtures, theoretical studies have shown that, in such a situation, domain formation can either occur in the nonadhering regions or in the adhering regions but never in both (40,41). In experiments with pore-spanning membranes, which are in a sense analogous to NA/PA states, Lo domains were observed to be confined to the nonadhering regions of the membrane above the pores (42).

Nanopit shapes: limitations and generalizations

As described in Methods, throughout this study we have focused on nanopits with a smooth axisymmetric shape that satisfies certain conditions, as follows: the defining height function \(z_{np}(r)\), defined on the interval \(0 \leq r \leq R\), was taken to be (i) nondecreasing with only one inflection point in its domain, (ii) differentiable at least once, and satisfying (iii) \(z'_{np}(0) = 0\) and (iv) \(z''_{np}(R) = 0\). Condition (i) ensured that the nanopit has a simple shape with a single minimum, whereas conditions ii, iii, and iv ensured the smoothness of the shape, i.e., the absence of “kinks” on the nanopit surface. We found that the adhesion of membranes to any such nanopit presents a number of universal features, namely that CA states are always metastable (see Eq. 11), that NA states become unstable at a tension-independent critical adhesive strength (see Eqs. 12 and 16), that the instability of NA states can be continuous toward PA states or discontinuous toward CA states at low membrane tension if the nanopit shape satisfies certain conditions (see Eqs. 13 and 14), and, finally, that PA states become unstable discontinuously toward CA states at a tension-dependent adhesive strength (see Eqs. 15 and 17). If the nanopit shape “breaks” some of the conditions (i–iv), the adhesion of the membrane to the nanopit will no longer exhibit all these features, as discussed below.

First, let us consider a nanopit that breaks condition iii (i.e., that has a “kink” at its bottom) such as the cone-shaped nanopit displayed in Fig. 7 a. In this case, CA states are no longer (meta)stable always: in fact, CA states become strictly impossible, because the membrane cannot adapt its shape to the kink without rupturing or forming a defect. Increasing values of the adhesive strength \(|W|\) will therefore only lead to more deeply adhered PA states. For the particular case of a tensionless membrane in contact with a cone-shaped nanopit such as the one in Fig. 7 a, scale invariance of the bending energy implies that the shape and energy of the unbound segment are independent of the contact radius \(r^*\), and that, therefore, the adhesion process is governed by the competition between adhesion and bending in the bound segment, with the equilibrium condition \(|W| = 2\kappa_c M_{np}^2(r^*)\). The mean curvature of the nanopit at the contact line is given by \(M_{np}(r^*) = \sin\alpha/2r^*\), where \(\alpha\) is the angle of the cone with the horizontal. We therefore find \(r^* = \sin\alpha\sqrt{\kappa_c/2|W|}\), implying that a continuous increase of \(|W|\) leads to a continuous decrease of \(r^*\) (i.e., to more deeply adhered PA states) without ever reaching the CA state with \(r^* = 0\).
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**FIGURE 7** More general nanopit shapes (in black) that “break” some of the conditions that we imposed in the Methods: (a and b) nanopits may be nonsmooth and instead display a “kink,” which may be located (a) at the bottom, (b) at the rim, or elsewhere along the surface of the nanopit. (c) Nanopits may have “wiggly” shapes with more than one inflection point along their profile. (d) Nanopits may be nonaxisymmetric. In (a)–(d), the membrane is depicted in blue. To see this figure in color, go online.

Alternatively, a nanopit may break condition iv and have a “kink” at the rim, such as the cylindrical nanopit displayed in Fig. 7 b. In this case, we expect NA states to be always (meta) stable, i.e., that there will not be a critical adhesive strength above which the NA state becomes unstable. The reason is that, to adhere to the inside of the nanopit, the membrane will have to first bend into it (which implies both a bending and tension energy cost), without a concomitant gain in adhesion energy. There will therefore always be an energy barrier separating the NA state from the PA or CA states, independently of the adhesive strength of the substrate. We have used the numerical shooting method (described in Methods) to calculate the shape of a tensionless membrane when bending into a cylindrical nanopit with a 90° kink at the rim (see the blue line in Fig. 7 b). The membrane must develop an overhang to avoid the rim and still adhere to the walls. The state depicted corresponds to the top of the energy barrier, with a barrier height given by \( \approx 0.9 \times 8\pi\kappa \), of the order of several hundred \( k_B T \) for a typical bending rigidity. This energy barrier will be even higher for membranes under tension. However, the energy barrier will be smaller (but still nonzero) for shallower nanopits, either with nonvertical walls or with sufficiently short vertical walls, in which case the membrane will make initial contact with the bottom rather than the walls of the nanopit. Similar considerations will come into play if condition ii for the nanopit shape is broken, i.e., if the nanopit displays a kink somewhere between its bottom and its rim.

In Fig. 7 c, we display a nanopit with a “wiggly” shape that breaks condition i, in that its height function \( z_{np}(r) \) has more than one inflection point in its domain, namely three in this case. This implies that the principal curvature of the nanopit that is perpendicular to the contact line changes sign three times, instead of just once. Our results concerning the (meta)stability of CA states for all values of membrane tension and adhesive strength, as well as the existence of a tension-independent stability limit of the NA state, remain unchanged independently of the number of inflection points, because our derivation of Eqs. 11 and 12 depended only on the local shape of the nanopit at the bottom or at the rim. However, we can show that, in this case, two or more distinct (meta)stable PA states will coexist with each other and with the CA state at sufficiently large membrane tensions. This can be directly inferred, in the limit of large tension, from the same argument that led to Eq. 15, because, in this case, \( |W| \) will have \( n \) maxima as a function of \( r^* \) when the height function has \( 2n - 1 \) inflection points. The same conclusion can be reached, without recurring to the limit of large tension, for the special case of “stair-like” nanopits constructed from a smooth combination of cylindrical and toroidal segments, using the same arguments that led to Eqs. 17, 18, and 19. We note, however, that we have not rigorously established the relation between the number of inflection points and the number of coexisting PA states, at low tensions, for nanopits of general shape.

Finally, it is also possible to relax the condition of axisymmetry, as in the nonaxisymmetric example of Fig. 7 d. Both the numerical shooting method as well as the analytical small gradient approximation used in this work (see Methods) were based on the assumption of axisymmetry and are thus not applicable to such nanopits. We can, however, make some educated guesses. First, we still expect NA states to be, in general, stable for sufficiently low adhesive strength and to become unstable above a tension-independent critical adhesive strength. At the rim of a nonaxisymmetric nanopit, the curvature \( C_{rim} \) perpendicular to the line where the nanopit meets with the flat substrate is no longer a constant and instead varies along the length of this line. Given that the contact curvature condition in Eq. 3 is valid for nonaxisymmetric geometries, and that in the NA state the unbound membrane is completely flat with \( C_\perp \equiv 0 \), we may expect the NA state to become unstable for adhesive strengths above \( |W| = min(\kappa C_{rim}^2/2) \), i.e., to be governed by the weakest rim curvature along the length of the contact line between the nanopit and the flat substrate. Second, we may wonder whether the fact that CA states are always (meta)stable remains valid. It is tempting to speculate that it will, as long as the bottom of the nonaxisymmetric nanopit is smooth. However, it is also possible that the adhesive strength threshold for (meta)stability of the CA state is proportional to a measure of the local deviation from axisymmetry at the bottom of the nanopit, such as \( |W| \propto \kappa (M_8^2 - K_8) \), where \( M_8 \) and \( K_8 \) are the mean and Gaussian curvatures at the bottom of the nanopit, which would still result in \( |W| = 0 \) for axisymmetric...
CONCLUSIONS

In summary, we have shown that the adhesion of biomembranes to substrates topographically patterned with nanopits displays many universal features that are independent of the detailed shape of the nanopits. We have provided conditions for the stability of NA, PA, and CA states valid for a very general class of nanopit shapes, see Eqs. 11, 12, 13, 14, and 15, as well as for the special case of cylindrical nanopits with vertical walls, see Eqs. 16 and 17. These conditions can be applied as guidelines to the design and analysis of experiments involving vesicles, supported bilayers, or cells in contact with nanopits. Furthermore, we have examined the coarse-grained effect of many nanopits on membrane-substrate adhesion. The presence of nanopits couples the tension of the membrane to the effective adhesiveness of the substrate, which should display hysteresis in response to cycles of increasing and decreasing tension. Nanopits can lead to both an increase or a decrease in the effective substrate adhesiveness, depending on the relative values of the real adhesive strength $W$ of the substrate and the membrane tension $\Sigma$ (see Eq. 22). We have described in detail how our results relate to the existing experimental evidence for supported lipid bilayers and biological cells in contact with substrates topographically patterned with nanopits, and furthermore, we have proposed an explicit way to test our results in experiments using micropipette-held giant vesicles in contact with such substrates. We have also discussed how the presence of nanopits may affect the integrity of the membrane as well as the lateral organization of the different membrane components in the case of multicomponent membranes. Finally, we have pointed to exciting and, to our knowledge, new directions in the theoretical and experimental study of membrane adhesion to nanopits with more complex shapes.

As a final remark, it is worth noting that, besides nanopits, our results may be used to describe the entry and migration of membranes into cylindrical nanopores. In this case, PA states will become unstable toward growth of a membrane protrusion into the pore. The stability limit of the PA state will nevertheless still be given by Eq. 17. For a description of cell migration through micron-sized pores, however, deformations of the cellular cortex and active cytoskeletal forces as well as osmotic pressure effects are expected to become important.

AUTHOR CONTRIBUTIONS

J.A.-C. and D.E.D. designed the research. J.A.-C. performed the research. J.A.-C. wrote the first draft. J.A.-C. and D.E.D. discussed and edited the manuscript.

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