Several methods now exist to solvate lipid bilayer discoids at the scale of tens of nanometres. Due to their size, such nanodiscoids have a comparatively large boundary-to-area ratio, making them unusually well-suited to probing the effects of Gaussian curvature. Arguing that fluctuations in discoid size and shape are quenched on formation, we quantify the stability, in terms of size and shape, of near-solvation discoid-like flaps that are subject to thermal fluctuations. Using cryo-Electron Microscopy images of Styrene Maleic Acid stabilised discoids, we deduce that stable, saddle-like discoids (with high Gaussian curvature) can likely be solvated from bulk lamellar ($L_\alpha$) phase at moderate-to-high surface tensions ($>10^{-4}$ N/m). We then describe how such tension-controlled solvation can be used for both measuring, and fractionating membrane components according-to, the modulus of Gaussian rigidity $\bar{\kappa}$. Opportunities for investigating the effects of Gaussian curvature on membrane-embedded proteins, which can be co-solvated during the formation process, are also discussed.

I. INTRODUCTION

Several long-chain molecules have now been shown to solvate nanoscale lipid bilayer discoids, with two prominent examples being the copolymer Styrene Maleic Acid (SMA) [1–4] and the $\alpha$-helical lipoprotein APO-A1 [5–7]. The key feature of these molecules is their amphipathic structure: in the presence of a lipid bilayer, the hydrophobic groups interact with the acyl chains of the lipids, whilst the hydrophilic parts face the solvent, resulting in a solution of stable nanodiscoids (see Fig. 1). Significantly, such discoids have been shown to preserve the integrity of co-solvated transmembrane proteins, implying an important role for the purification, structural determination, and functional characterization of membrane proteins [7–14].

The modulus of Gaussian rigidity, $\bar{\kappa}$, quantifies a membrane’s preference for forming locally saddle-like or spherical shapes, and determines the equilibrium topology of bulk membrane phases, e.g., a highly connected sponge ($L_3$), one or more (large) membranes ($L_\alpha$) or a large number of small vesicles [15]. However, $\bar{\kappa}$ is notoriously difficult to measure. This is because of the Gauss-Bonnet theorem [15–17], which states that Gaussian curvature doesn’t couple to any observable deformation modes for one component membranes of fixed, boundary-free topology. Even for membranes with a boundary (or a hole [18]), the coupling to Gaussian curvature is usually negligible for all but the largest boundary-to-area ratios. Solvated nanodiscoids have an extremely large boundary-to-area ratio, making them not only technologically important, but also uniquely suited for studying the role of $\bar{\kappa}$ in membranes.

In this article we study how lipid nanodiscoids can be used to probe phenomena that couple to Gaussian curvature. We show how they can be used to both indirectly measure $\bar{\kappa}$, and fractionate lipids by $\bar{\kappa}$ into discoids with negative Gaussian curvature. More generally, given the extreme values of Gaussian curvature, both positive and negative, found in vesicles and organelles associated with protein synthesis and sorting [19, 20], it is natural to ask: what are the effects of this curvature on membrane-embedded proteins, and does it play a role in sorting. In this context, lipid nanodiscoids may have a wider role as a tool for investigating the effects of Gaussian curvature on co-solvated transmembrane proteins.
FIG. 2. (Color online) Cartoon of the putative formation process. Driven by its amphiphilic character, the solvating long-chain molecule inserts into the membrane (a). The presence of the solvating compound leads to a hole and a free edge (red dashed line) in the membrane. The high energetic cost of such free edges, however, drives the closure of such holes, first forming a straight ‘cut’ and then bringing the two ends of the solvating molecule together (b). Near solvation, a small membrane flap remains (b and c) which can still exchange molecules with the bulk membrane. On ‘pinching-off’, the differences in flap size and shape that result from such fluctuations become quenched, since the elastic modulus of membranes held at fixed particle number is extremely large. The result is a polydisperse mixture of solvated nanodiscoids (d).

II. POLYDISPERSITY QUENCHED ON FORMATION

We begin with the observation that solvated nano-scale discoids form a relatively polydisperse mixture, with an apparently broad range of areas and shapes. Since lipid bilayers are essentially incompressible, we conclude that such variation is a result of the formation process, which is poorly understood. Moreover, we note that the timescales of dissipation, due to either the viscosity of the bilayer, or the surrounding water, are comparatively short. We therefore assume a quasi-static description of nanodisc solvation, which we use to quantify the statistics of area and shape variation, and hence imply the aforementioned new role for nanodiscoids in the context of the probing of Gaussian curvature.

Neglecting any potential complications associated with adsorption/insertion, our starting point is a fully-inserted protein/co-polymer restricted to planar self-avoiding conformations (i.e., crossings are not allowed). It is assumed that the (known) amphipathic properties of the long-chain solvating molecule then lead the formation of a flap of bilayer, able to protrude discontinuously from the membrane (see Fig. 2). The large energy-per-unit-length associated with an unadorned bilayer edge then ensures that the “hole” left behind by such a flap quickly becomes a “cut”, which effectively runs in a straight line between the two ends of the solvating protein/co-polymer (see red and red-dotted lines in panels a, b and c of Fig. 2). Further energy minimization then drives the meeting of the two free ends, reducing the cut length, and leading to discoid solvation. Very close to formation, when the cut is very small, an effective line tension can be associated with the closed curve comprising the boundary of the near-solution flap and the cut itself.

Provided that it is very near the point of “closure” (when the protein/co-polymer ends meet) and that it retains the structure (and symmetry) of a standard bilayer, a Helfrich-like continuum theory [16] can be used for the bulk of the flap. Assuming uniform lipid composition, the leading order energetics [21] are related to the geometry of the midsurface $S$ of the discoid-like flap via

$$
\mathcal{H}_m = \int_S dA \left[ \sigma + \frac{\kappa}{2} \left( 2H - c_0 \right)^2 + \kappa K \right],
$$

where $\sigma$ is a surface tension, set by exchange of lipids through the almost-closed gap between protein/co-polymer ends. As usual, $H$ and $K$ are the mean and Gaussian curvatures, and $\kappa$ is the membrane bending rigidity. We assume that there is no density mismatch between bilayer leaflets and therefore take $c_0 = 0$ throughout.

There is also an energy associated with boundary, $\partial S$, which is the closed line formed by concatenating the solvating protein/co-polymer and the remaining small cut. We include both a line tension $\tau$, and an energetic cost to bending (amphipathic asymmetry precludes any twist). By symmetry, the latter must be invariant under sign change of both the normal and geodesic curvatures of $\partial S$, for which we use the symbols $q_n$ and $q_g$, respectively. The contribution is written as the following line integral

$$
\mathcal{H}_b = \int_{\partial S} dl \left[ \tau + \frac{k_g}{2} (q_g - q_0)^2 + \frac{k_n}{2} q_n^2 \right],
$$

where $k_g$ and $k_n$ are effective bending moduli. The former corresponds to in-tangent-plane bending, whilst the
latter corresponds to out-of-tangent-plane bending. For definitions of the relevant geometrical quantities, see the Supplementary Material (SM). Notice that since the solvating protein/co-polymer is assumed to preserve the symmetry of the bilayer on reflection about \( S \) (true for both SMA and APO-A1), a non-zero spontaneous line curvature \( g_0 \) is only permitted in the tangent plane of the surface, and not in the normal direction.

Of course, once solvated, lipid exchange with the membrane (reservoir) is no longer possible. All subsequent area and shape fluctuations are then suppressed by the disproportionately large intrinsic elastic modulus of lipid bilayers (when held at fixed particle number) and, as a result, any thermally-induced fluctuations in area due to lipid exchange when close to solvation become fixed, or “quenched”, for all time at the point when the discoid finally detaches.

III. TENSION CONTROLLED INSTABILITY TO PRINGLE SHAPES

To understand the ramifications of these ideas, consider flat shapes (i.e., \( H = K = q_n = 0 \)). In this case, the total energy, \( \mathcal{H} = \mathcal{H}_b + \mathcal{H}_m \), is minimised by discs of radius \( R_0 \), which is related to the line tension \( \tau \) (and \( k_g \), \( q_0 \) and \( \sigma \)) via

\[
\tau = \frac{k_g}{2R_0^2} (1 - q_0^2 R_0^2) - R_0 \sigma, \quad (3)
\]

(see SM for details). The in-plane stability can be investigated by writing an angle-dependent radius \( R(\theta) = R_0 + \delta R(\theta) \) such that \( \delta R(\theta) = \epsilon R_0 \sum_n \Re [A_n \exp(i n \theta)] \), where \( \Re \) indicates the real part, \( A_n \subset \mathbb{C} \), \( |A_n| \leq 1 \) for all \( n \in \mathbb{N} \), and \( \epsilon \ll 1 \) is dimensionless. The energy, \( \mathcal{H} \), can then be expanded as a power series in terms of \( \epsilon \). Here, the mean-squared thermal amplitude of the \( n \)th fluctuation mode follows by the principle of equipartition of energy (see SM) \([23]\)

\[
\epsilon^2 \langle |A_n|^2 \rangle = \frac{k_g T R_0}{\pi (n^2 - 1) [k_g (n^2 - 1) - 3 \sigma^2]}.
\]

The \( n = 0 \) mode is a dilation, and is always stable (we expect \( \sigma, k_g > 0 \) for all lipid discs, irrespective of whether they are cut from living cells, adsorbed bilayers or vesicles). The \( n = 1 \) mode corresponds to a translation where the disc remains circular to lowest order, and is of indeterminate amplitude. In general, for \( n > 1 \), a mode of degree \( n \) is stable if \( k_g \geq R_0^2 \sigma / (n^2 - 1) \). This puts a lower bound \( k_g \geq R_0^2 \sigma / 3 \) on the geodesic rigidity of the attached solvating compound that is required for stable circular discs.

For non-flat discoids, we consider the subset of all out-of-plane deformations that can be parametrised by the
and extracting the mean-squared deviation of the semi-major axes (cally) much smaller than the membrane correlation length \( \kappa \) and two (orthogonal) principal curvatures at the centre, \( c_1 \) and \( c_2 \). This is reasonable provided that the disc is (laterally) much smaller than the membrane correlation length \( \sqrt{\kappa/\sigma} \), which is typically at least a few tens of nm. The curvatures are labelled, without loss of generality, such that \( |c_1| \geq |c_2| \). Using a polar Monge approach, the height field is chosen to be

\[
h(r, \theta) = \frac{\alpha r^2}{4 R_0} \left[1 + \phi + (1 - \phi) \cos 2\theta\right],
\]

where the angle \( \theta \) is measured from the direction of \( c_1 \), \( \phi = c_2/c_1 \) takes values in the interval \([-1, +1]\), and \( \alpha = R_0 c_1 \ll 1 \) is a small dimensionless parameter. Both mean and Gaussian curvatures remain constant across the disc up to \( O(\alpha^2) \) making (1) straightforward to calculate (see SM). However, this is not the case for the either line element at the boundary \( dl \) or the line curvatures \( q_0 \) and \( q_n \) appearing in (2). Indeed, both \( dl \) and \( q_n \) also rely on \( \epsilon \), and we therefore adopt a notation where the \( O(\alpha^2 \epsilon^2) \) term in an expansion of \( \mathcal{H} \) is written as \( \alpha^2 \epsilon^2 \mathcal{H}^{(a,\epsilon)} \). The resulting expression (see SM) has neither any first order terms \( \text{i.e., at } O(\alpha) \text{ or } O(\epsilon) \), nor any \( O(\alpha \epsilon) \) cross terms:

\[
\mathcal{H} = \mathcal{H}^{(0,0)} + \epsilon^2 \mathcal{H}^{(0,2)} + \alpha^2 \mathcal{H}^{(2,0)} + O\left(\epsilon^3\right) + O\left(\alpha^3\right).
\]

The coefficients \( \mathcal{H}^{(0,0)} \) and \( \mathcal{H}^{(0,2)} \) can therefore be read-off from the previously calculated energy of a flat disc \( \tau \) is unchanged from (3)]. Moreover, the earlier stability analysis holds too, but now refers to the projected disc size, which is independent of out-of-plane perturbations to \( \epsilon^2 \) and \( \alpha^2 \).

The stability of out-of-plane perturbations is governed by the sign of \( \mathcal{H}^{(2,0)} \), which depends not only on \( \phi \), but also the material properties of the solvating polymer \( (R_0, k_g, k_n, \text{and } q_0) \), the membrane bending moduli \( (\kappa \text{ and } \bar{\kappa}) \), and the membrane tension \( \sigma \), which is set during formation. We focus on how the properties of the membrane (i.e., tension and bending) affect discoid stability and, in particular, the role of \( \bar{\kappa} \).

Leaving the details to the SM, we write \( \mathcal{H}^{(2,0)} = \pi \phi \left[ \bar{\kappa} - \bar{\kappa}^* (\phi) \right] \), which introduces a critical value of Gaussian rigidity, \( \bar{\kappa}^* \). Fig. 3 shows how this quantity determines the stability of a given perturbation \( \phi \) as a function of the ratio \( \bar{\kappa}/\kappa \). Also shown is the magnitude of \( \mathcal{H}^{(2,0)} \), and hence the rate at which the perturbation grows or shrinks in time. For a band of values of \( \bar{\kappa}/\kappa \), all perturbations are suppressed and only flat discs are stable. Outside of this band, perturbations characterised by a range of \( \phi \) are unstable. However, the dominant (i.e., fastest growing) mode always corresponds to either \( \phi = -1 \) or \( \phi = +1 \). That is, a saddle with principal curvatures of equal magnitude—a “pringle”—or a shallow section of a sphere, respectively. In the case of the former, the instability that takes a flat disc to a pringle is just \( \bar{\kappa} \geq \bar{\kappa}^* \), where the critical value

\[
\bar{\kappa}^* = \left( \frac{3}{2 R_0} - q_0 \right) k_g + \frac{k_n}{2 R_0} - \frac{R_0^2 \sigma}{4},
\]

is marked in Fig. 3. For a given \( \bar{\kappa} \) therefore, pringles are always unstable for tensions above some critical value \( \sigma_p \), which can be found by inverting (7).

We can assess whether such tensions \( \sigma \geq \sigma_p(\bar{\kappa}) \) are physically plausible by analysing cryo-Electron Microscopy images of SMA stabilised discoids. When combined with equipartition (4), such images permit the calculation of an effective upper bound on \( \sigma_p \) by estimating the maximum possible values of \( k_g \) and \( k_n \). Figure 4 shows one such cryo-EM image, prepared according to (4)—i.e., stabilised by SMA and cut from moderate tension vesicles \((\sigma \sim 10^{-4} \text{ N/m})\) that were synthesised from E. Coli lipid extract \((\bar{\kappa} \sim -\kappa \text{ [22])}\). For such systems, if \( k_g \) is sufficiently large to stabilise the fluctuations in projected discoid shape and size \( (\geq R_3^3 \sigma/3) \), then out of plane fluctuations are also suppressed if \( k_n \geq k_g \). (That is, the line \( \bar{\kappa} = -\kappa \) is within the grey region of Fig. 3). In
this case, equilibrium discoids are flat discs, and any variation in shape appearing in Fig. 1 is a result of projection onto the focal plane. Using image processing, each discoid may be fitted to an ellipse, and the mean-squared deviation of the semi-major axes may be calculated (Fig. 1 and SM). The result can be used to invert Eq. (4) at \( n = 0 \) and predict an upper estimate for the geodesic rigidity of \( k_g \). Further assuming \( k_g = k_n \) and setting \( q_0 = 1/R_0 \), the aforementioned upper estimate for \( k_g \) yields an upper bound on \( \sigma_p \) of \( \sim 10^{-2} \) N/m, a tension at which bilayer membranes typically rupture [13]. We deduce, therefore, that pringle-shaped discoids can likely be cut from \( L_\alpha \) phase membrane at large, but physically plausible, tensions. Moreover, by considering higher-order contributions to the energy, it can be shown that the resultant pringles are stable for moduli of Gaussian rigidity \( \tilde{\kappa} \geq \max (\tilde{\kappa}_p, \tilde{\kappa}_\chi) \) (see SM), where \( \tilde{\kappa}_p \) a critical value similar to that of Eq. (7), but which is determined at \( O (\alpha^4) \).

IV. FRACTIONATION ACCORDING TO MODULUS OF GAUSSIAN CURVATURE

Significantly, the pringle transition can occur before the bulk membrane phase switches from lamellar (\( L_\alpha \)) to sponge (\( L_3 \) [5]). That is, saddle-shaped discoids can form spontaneously after being cut from flat sheets, as opposed to being cut from a bilayer with equilibrium negative Gaussian curvature, such as the sponge phase. Stable pringles are interesting because they promote the preferential sorting of lipids with larger intrinsic Gaussian rigidities into the discoids as they are formed. To illustrate this we consider a simple two-component model in which an SMA stabilised pringle emerges from a well-mixed (bulk) membrane having an average Gaussian rigidity \( \tilde{\kappa} \) in the regime \( \tilde{\kappa} > \max (\tilde{\kappa}_p, \tilde{\kappa}_\chi) \). Relative to the bulk membrane, the pringle may contain an additional area fraction \( \psi \) of one of the components, resulting in a Gaussian rigidity for the pringle of the form \( \tilde{\kappa} + \delta \tilde{\kappa} \psi \), with \( \delta \tilde{\kappa} = \tilde{\kappa}_1 - \tilde{\kappa}_2 > 0 \) the difference between the Gaussian rigidity of each component. The membrane Hamiltonian might then include an extra term

\[
H_{\psi} = \int_S \frac{\chi \psi^2}{2} dA,
\]

with \( \chi \) a Flory parameter that approaches zero as the bulk membrane approaches the demixing transition. The inclusion of (8) implies that the disc spontaneously adopts the shape of a stable pringle, with curvature given by

\[
|c_1| = \frac{1}{R_0} \left[ \frac{2}{3} \frac{(\tilde{\kappa} - \tilde{\kappa}_p)}{(\tilde{\kappa} - \tilde{\kappa}_\chi)} \right]^{1/2},
\]

when \( \tilde{\kappa} > \max (\tilde{\kappa}_p, \tilde{\kappa}_\chi) \) for \( \tilde{\kappa}_p = \tilde{\kappa}_p - 2(\delta \tilde{\kappa})^2/\chi R_0^2 \) (see SM). The corresponding equilibrium composition of the discoid is characterised by the additional area fraction

\[
\psi = \frac{2 \delta \tilde{\kappa} \left( \tilde{\kappa} - \tilde{\kappa}_p^* \right)}{3 \chi R_0^2 \left( \tilde{\kappa} - \tilde{\kappa}_\chi^* \right)},
\]

which is occupied by lipids with modulus of Gaussian rigidity \( \tilde{\kappa}_1 \).

V. CONCLUSIONS AND DISCUSSION

Taken as a whole, our analysis suggests the possibility of using lipid nanodiscoids to not only phase-separate, but actually fractionate membrane components by \( \tilde{\kappa} \); those components with the largest values of \( \tilde{\kappa} \) will be preferentially sorted into pringles. It is also possible that the instability described here could be used as the basis of a technique to measure or compare values of \( \tilde{\kappa} \). One possible approach is to slowly increase the surface tension of vesicles via micropipette-aspiration or osmotic control: saddle shapes should first form when \( \tilde{\kappa} = \tilde{\kappa}_p^*(\sigma) \). We reiterate that these results are especially pertinent given the lack of available alternatives that prob lipid coupling to membrane Gaussian curvature.

We propose that such ideas can readily be extended to areas of significant Biological interest: the localisation or activities of membrane-bound proteins may also depend on Gaussian curvature via both the protein’s effective shape and elastic response to deformation. Indeed, given that membrane-bound proteins can be co-solvented with their integrity preserved, the nanoscale discoids described here provide an interesting new quantitative technology for studying the wider role of Gaussian curvature in cell Biology—e.g., the sorting and function of membrane-bound proteins in organelles whose function is closely tied to morphology, such as the Endoplasmic Reticulum or Golgi Apparatus. We therefore welcome further work in the area.

Acknowledgments

R.G.M and M.S.T acknowledge the support of EPSRC grant # EP/E501311/1, a Leadership fellowship to M.S.T. R.G.M also acknowledges the Simons Foundation and the Tata Institute of Fundamental Research. T.R.D acknowledges the support of a BBSRC BRIC project grant # BB/G010412/1.

Author contributions

M.S.T and T.R.D conceived-of and designed the research. R.G.M and M.S.T performed analysis and T.R.D contributed images/data. R.G.M and M.S.T wrote the manuscript in consultation with T.R.D.
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SUPPLEMENTARY MATERIAL

INTRODUCTION

The purpose of this supplementary material is threefold. First, to provide any necessary theoretical background (in a terse, but self-contained way), particularly with respect to differential geometry (for further details, the reader is referred to [17, 24]). Second, to provide a detailed account (making heavy use of the aforementioned background) of the calculations whose results appear in the main manuscript. Lastly, to describe the protocol used to analyse the cryo Electron Microscopy images.

GEOMETRY

Setup and notation

Let \( S \) be a smooth Reimannian manifold representing the nanodiscoid midsurface. If points on the midsurface are labelled by an “internal” coordinate \( u \in \mathbb{R}^2 \), then \( S \) is just the image of \( u \) under an embedding \( F : \mathbb{R}^2 \to \mathbb{R}^3 \). That is, the position in \( \mathbb{R}^3 \) of a point \( u \) is given by the vector \( R(u) =: F(u) \).

In terms of notation, the convention, which will be used throughout, is that Greek indices take values 1 or 2, whilst Latin indices take 1, 2 or 3. Bold typeface is used to represent vectors in \( \mathbb{R}^3 \), normalised basis vectors, independent of position \( R(u) \). By contrast, an overarrow is used for vectors in \( \mathbb{R}^2 \), such that \( \vec{v} = v^\alpha \hat{e}_\alpha \), with \( \{ \hat{e}_\alpha : \alpha = 1, 2 \} \) representing non-normalised basis vectors, which depend on position \( u \).

The embedding \( F \) corresponds to a pushforward \( F_* : T_u \mathbb{R}^2 \to T_{R(u)} \mathbb{R}^3 \), whose operation is defined by:

\[
F_* (\text{e}_\alpha) = \frac{\partial R(u^\alpha)}{\partial u^\alpha}.
\]  

(11)

The notion of \( F_* (\hat{e}_\alpha) \) can be used to define the unit normal to \( S \):

\[
\hat{n} = \frac{F_* (\hat{e}_1) \times F_* (\hat{e}_2)}{|F_* (\hat{e}_1) \times F_* (\hat{e}_2)|}
\]  

(12)

where the symbol \( \times \) represents the usual cross product in \( \mathbb{R}^3 \). (For the purposes of this article, it suffices to assume that the orientation of the basis of a manifold is the same as the orientation of the manifold itself).

Forms

A form \( r^{(k)} \) of degree \( k \) is a linear functional that takes \( k \) tangent vectors and returns a real number. For example, a 0-form is a function, a 1-form is a co-vector, and higher forms of degree \( k \) are tensors of rank \((0,k)\). (For notation, the degree of a form will be indicated by a bracketed superscript unless explicitly stated otherwise).

1-forms

At a point \( u \), the basis of 1-forms that spans the cotangent space, \( T_u^* \mathbb{R}^2 \), is given by \( \{ du^\alpha : \alpha = 1, 2 \} \), such that \( du^\alpha (\hat{e}_\beta) = \delta_\beta^\alpha \), where \( \delta_\beta^\alpha \) is the Kronecker delta symbol. In this basis, \( p = p^\alpha du^\alpha \), where once again there is an implicit sum over repeated indices.

For example, consider a curve \( C \), parameterised by \( t \) and embedded in \( S \). \( C \) has tangent vectors \( T = \partial R/\partial t \) with pre-image \( \hat{T} \) under \( F_* \). In this case, the line element “\( dl \)” is just

\[
dl := \| \hat{T} \| dt = \left[ g_{\alpha\beta} T^\alpha T^\beta \right]^{1/2} dt.
\]  

(13)

Equivalently, “\( dl \)” can also be defined relative to the Euclidean coordinates of the embedding space:

\[
dl = \left\{ \delta_{ij} \left[ F_* (\hat{T}) \right]^i \left[ F_* (\hat{T}) \right]^j \right\}^{1/2} dt,
\]  

(14)

where the implied sum is now over \( i,j = 1, 2, 3 \) and not \( \alpha, \beta = 1, 2 \) as in \( (13) \) and \( \delta_{ij} \) is just the metric in Euclidean coordinates.
First fundamental form, metric and inner product

The embedding function $F$ induces a metric on $\mathbb{R}^2$ via the pullback $F^*$. That is $g_{\alpha \beta}(u) = (F^* I)(\tilde{e}_\alpha, \tilde{e}_\beta) = F_*(\tilde{e}_\alpha) \cdot F_*(\tilde{e}_\beta)$, where $I$ is the first fundamental form of $\mathbb{R}^3$ (i.e., with coefficients $\delta^{ij}$) and “$\cdot$” is the usual dot product. At each point $u \in \mathbb{R}^2$, the induced metric can be used to define an inner product $\langle \cdot, \cdot \rangle : T_u \mathbb{R}^2 \times T_u \mathbb{R}^2 \to \mathbb{R}$.

That is, for arbitrary vectors $\vec{v}$ and $\vec{w}$, we define $\langle \vec{v}, \vec{w} \rangle := v^\alpha g_{\alpha \beta} w^\beta$. Such an inner product permits the explicit identification of a vector, e.g., $\vec{v}$, with its dual 1-form, $v^\alpha$, by the condition $v(\vec{w}) = \langle \vec{v}, \vec{w} \rangle$, which holds for all $\vec{w}$. Noticing that $v(\vec{w}) = v_\alpha d\nu^\alpha(\vec{w}) = v_\alpha w^\alpha$ and using the above definition of the inner product of two vectors implies the raising and lowering properties of the metric and its inverse $[g^{\alpha \beta} = (g_{\alpha \beta})^{-1}]$, respectively. That is, $v_\alpha = g_{\alpha \beta} v^\beta$ and $v^\alpha = g^{\alpha \beta} v_\beta$. Using this property, the inner product acting on two 1-forms can be defined in a complementary way to that of the inner product on vectors:

$$\langle v, w \rangle := v_\alpha g^{\alpha \beta} w_\beta = v_\alpha w^\alpha = (\vec{v}, \vec{w}).$$  \hfill (15)

Covariant derivative

The covariant derivative at a point $u$ on $S$ is a generalisation of the directional derivative. It calculates the rate of change of a tensor field (at $u$) whilst moving along the unique geodesic that has tangent vector with pre-image $\vec{y}$ (at $u$) under $F_*$.  

Scalars

The action of the covariant derivative on a scalar field $\phi$ is defined to be

$$\nabla_\vec{y} \phi := \phi_\alpha d\nu^\alpha(\vec{y}),$$  \hfill (16)

where a subscript comma “,” is shorthand for a partial derivative, i.e., $\phi_\alpha := \partial \phi / \partial u^\alpha$.

Vectors

When acting on a tangent vector $\vec{v} = v^\alpha \tilde{e}_\alpha$, we write

$$\nabla_\vec{y} \vec{v} := \tilde{e}_\alpha (v^\alpha ; \beta) d\nu^\beta(\vec{y}),$$  \hfill (17)

where the components $v^\alpha ; \beta$ are given by

$$v^\alpha ; \beta := v^\alpha, \beta + \nu^\gamma \Gamma^\alpha_{\beta \gamma}.$$  \hfill (18)

Once again, a subscript comma “,” is shorthand for a partial derivative, $v^i, j := \partial v^i / \partial u^j$, whilst the $\Gamma^\alpha_{\beta \gamma} = g^{\alpha \delta} (g_{\delta \gamma, \beta} + g_{\delta \beta, \gamma} - g_{\beta \gamma, \delta})/2$ are Christoffel symbols, which define the action of the covariant derivative, via $\nabla_\vec{e}_\alpha \vec{e}_\beta = \tilde{e}_\gamma \Gamma^\gamma_{\alpha \beta}$. Note that the shorthand $\nabla_\alpha := \nabla_\vec{e}_\alpha$ is frequently used in physics.

1-forms

For a 1-form, the action of the covariant derivative can be defined by demanding that the “Leibniz rule” holds. That is, if a scalar field is defined by the action of a 1-form on a vector, i.e., $\phi := v(\vec{w}) = v^\alpha w_\alpha$, then

$$\nabla_\vec{y} (v^\beta w_\beta) = (v^\beta w_\beta)_\alpha := v^\beta, \alpha w_\beta + v^\beta w_{\beta, \alpha}.$$  \hfill (19)

The result is that $v_{\alpha ; \beta} := v_{\alpha, \beta} - v_\gamma \Gamma^\gamma_{\alpha \beta}$, which is consistent with the notion of using the metric as a raising / lowering operator (i.e., $v_{\alpha; \beta} = g_{\alpha \gamma} \nu^\gamma ; \beta$). In coordinate free notation, this is equivalent to

$$(\nabla_\vec{y} v)(\vec{w}) := \nabla_\vec{y} [ v(\vec{w}) ] - v (\nabla_\vec{y} \vec{w}) .$$  \hfill (20)
Consider the derivative
\[
\frac{\partial \hat{n}}{\partial u} = \hat{n}_{,\alpha},
\]
i.e., the rate (and direction) of change in the unit normal to \( S \) as \( u^\alpha \) is varied, expressed as a vector in \( \mathbb{R}^3 \). Since \( \hat{n} \) is a unit vector, the result must still be tangent to \( S \) and therefore
\[
\hat{n}_{,\alpha} = -b^\beta\alpha \mathbf{F}_\alpha(\hat{e}_\beta),
\]
which is known as the Weingarten equation (the assignment of a minus sign being convention). Given the right-hand side, we can use the coefficients from the above to construct a linear map \( b : T\mathbb{R}^2 \rightarrow T\mathbb{R}^2 \) by writing \( \tilde{b}(\tilde{v}) = -u^\beta \hat{e}_\alpha b^\alpha\beta \), for arbitrary \( \tilde{v} \). Similarly, there is a natural bilinear form \( II \), known as the second fundamental form, that can be associated with such a map, whose action is given by
\[
II(\tilde{v}, \tilde{w}) = \left\langle \tilde{v}, \tilde{b}(\tilde{w}) \right\rangle = v^\gamma w^\beta \left( \hat{e}_\gamma, -b^\alpha\beta \hat{e}_\alpha \right).
\]
That is
\[
II = -[\mathbf{F}_\alpha(\hat{e}_\alpha) \cdot \hat{n}_{,\beta}] \ du^\alpha \otimes du^\beta = b_{\alpha\beta} \ du^\alpha \otimes du^\beta,
\]
where \( b_{\alpha\beta} = g_{\alpha\gamma} b^\gamma\beta = -[\mathbf{F}_\alpha(\hat{e}_\alpha) \cdot \hat{n}_{,\beta}] \). Notice that since \( \partial [\mathbf{F}_\alpha(\hat{e}_\alpha) \cdot \hat{n}] / \partial u^\beta = 0 \), we have \( b_{\alpha\beta} = (\partial \mathbf{F}_\alpha(\hat{e}_\alpha) / \partial u^\beta) \cdot \hat{n} \). More generally, the derivative of basis vectors \( \hat{e}_\alpha \) with respect to some coordinate \( u^\beta \) on \( S \) can be decomposed into tangent and normal parts.
\[
\frac{\partial \mathbf{F}_\alpha(\hat{e}_\alpha)}{\partial u^\beta} = \Gamma^\gamma_{\alpha\beta} \mathbf{F}_\gamma(\hat{e}_\gamma) + b_{\alpha\beta} \hat{n},
\]
which is known as Gauss’ equation.

**Curvature**

**Lines**

In \( \mathbb{R}^3 \), the curvature \( q \) of a line \( \gamma(t) \) at a given point \( x \) is just the norm of the covariant derivative acting on the unit tangent to the line (in the direction tangent to \( \gamma \)) at \( x \):
\[
q := \| \nabla_T \hat{T} \| = \left[ \delta_{ij} \left( \nabla_T \hat{T} \right)^i \left( \nabla_T \hat{T} \right)^j \right]^{1/2},
\]
where
\[
\hat{T} = \frac{d\gamma}{dt},
\]
are just normalised tangent vectors to \( \gamma(t) \) and the covariant derivative reduces to the usual directional derivative of \( \mathbb{R}^3 \), i.e., \( \hat{T} \cdot (\partial/\partial x, \partial/\partial y, \partial/\partial z)^T \).

For lines that are also embedded in a sub-manifold (e.g., the boundary \( \partial S \) is a line in both \( \mathbb{R}^3 \) and \( S \)) there are two common measures of curvature: geodesic and normal. The geodesic curvature is a measure of curvature in the tangent plane \( TS \). That is
\[
q_g := \| \nabla_T \hat{T} \| = \left[ g_{\alpha\beta} \left( \nabla_T \hat{T} \right)^\alpha \left( \nabla_T \hat{T} \right)^\beta \right]^{1/2},
\]
where \( \hat{T} \) is the pre-image of \( \hat{T} \) under \( F_* \). By contrast to \( \| \hat{T} \| \), the normal curvature measures the out-of-(sub)manifold curvature and is given by
\[
q_n := \left[ \delta_{ij} \left( \left( \nabla_T \hat{T} \right) \cdot \hat{n} \right) \left( \left( \nabla_T \hat{T} \right) \cdot \hat{n} \right) \right]^{1/2}.
\]
Both \( q_g \) and \( q_n \) are linked to \( q \) via the following relation
\[
q = \sqrt{q_g^2 + q_n^2}.
\]
Surfaces

At a given point \( p \in \mathbb{R}^2 \), each unit vector \( \hat{\gamma} \) corresponds to a unique curve \( C \) on \( S \) that also lies in the plane \( P \) spanned by \( n \) and \( F_\gamma (\hat{\gamma}) \). The action of the second fundamental form on a given \( \hat{\gamma} \), results in the normal curvature \( c^{(n)}_\gamma \) of \( S \) in the direction of \( F_\gamma (\hat{\gamma}) \) (i.e., the curvature of \( C \) in \( P \)). We write,

\[
\Pi(\hat{\gamma}, \hat{\gamma}) = \pm c^{(n)}_\gamma,
\]

where “+” indicates whether \( C \) is curving towards the unit normal, and vice-versa for “-”. Since the normal curvature will change dependent on which direction \( \hat{\gamma} \) is chosen, we define the principal directions:

\[
\hat{\gamma}_1(p) = \arg\max_{\hat{\gamma} \in T_p \mathbb{R}^2} \Pi(\hat{\gamma}, \hat{\gamma}), \quad \text{and} \quad \hat{\gamma}_2(p) = \arg\min_{\hat{\gamma} \in T_p \mathbb{R}^2} \Pi(\hat{\gamma}, \hat{\gamma}).
\]

The principal curvatures are then given by

\[
c_\alpha(p) = \Pi(\hat{\gamma}_\alpha, \hat{\gamma}_\alpha), \quad \forall \alpha = 1, 2.
\]

It can be shown that the \( c_\alpha \) are eigenvalues of the linear operator \( \vec{b} \) from \[ 4 \] That is,

\[
\vec{b}(\hat{\gamma}_\alpha) = c_\alpha \hat{\gamma}_\alpha,
\]

where if \( c_1 \neq c_2 \), the principal directions are orthogonal. We may now define two different types of local curvature of \( S \): the mean curvature

\[
H := \frac{1}{2} \text{Tr} b^\alpha_\beta = \frac{1}{2} \text{Tr}_g \Pi = \frac{c_1 + c_2}{2},
\]

and the Gaussian curvature

\[
K := \text{Det} b^\alpha_\beta = \frac{\text{Det} h_\alpha^\beta}{\text{Det} h_\alpha^\beta} = c_1 c_2.
\]

SOLVATED MEMBRANE NANODISCOIDS

Consider a nanoscale bilayer discoid, stabilised by a solvating compound such as Styrene Maleic Acid or the lipoprotein APO-1A. Write \( \mathcal{H} = \mathcal{H}_m + \mathcal{H}_b \), where

\[
\mathcal{H}_m = \int_S dA \left[ \sigma + \frac{\kappa}{2} (2H - c_0)^2 + \bar{\kappa}K \right],
\]

and

\[
\mathcal{H}_b = \int_{\partial S} dl \left[ \tau + \frac{k_g}{2} (q_g - q_0)^2 + \frac{k_n}{2} q_n^2 \right],
\]

are energetic contributions from the bilayer membrane and solvating compound, respectively. In the first integral \[ 37 \], \( dA \) is the surface area element of \( S \), \( H \) and \( K \) are the mean \[ cf. Eq. (35) \] and Gaussian \[ cf. Eq. (36) \] curvatures respectively, and \( \sigma \) is a surface tension. The moduli \( \kappa \) and \( \bar{\kappa} \) are the bending and Gaussian (saddle-splay) rigidities, respectively, and \( c_0 \) is the membrane’s spontaneous (mean) curvature. In the second integral \[ 38 \], \( dl \) is the line element \[ cf. Eq. (13) \] of the surface boundary \( \partial S \), \( \tau \) is a line tension, \( q_g \) is the geodesic curvature \[ cf. Eq. (28) \], and \( q_n \) is the normal curvature \[ cf. Eq. (29) \]. The coefficients \( k_g \) and \( k_n \) are corresponding bending moduli with dimensions of energy multiplied by length. The SMA co-polymer is assumed to have spontaneous (line) curvature \( q_0 \) in the tangent plane to the disc, but not in the normal direction (i.e., it preserves the up-down symmetry of the bilayer). Restricting the analysis to surfaces with an Euler characteristic of one, the Gauss-Bonnet theorem gives:

\[
\int_S K dA = \int_{\partial S} k_g dl + 2\pi.
\]

Substituting into \[ 37 \] and \[ 38 \], we have

\[
\mathcal{H} = \int_S dA \left[ \sigma + \frac{\kappa}{2} (2H - c_0)^2 + \bar{\kappa}K \right] + \int_{\partial S} dl \left[ \tau + \frac{k_g}{2} q_g^2 + \frac{k_n}{2} q_n^2 \right] - k_g q_0 2\pi.
\]

where \( \bar{\kappa}_k := \bar{\kappa} + q_0 k \) and \( \tau_k := \tau + k q_0^2/2 \) are re-normalised constants.
Assume that $H = K = c_0 = q_n = 0$, which implies $S$ is just some bounded domain in $\mathbb{R}^2$ and only the line integral of $\mathbf{q}$ needs to be calculated. Using polar coordinates $(r, \theta)$ it is assumed that the discoid boundary $\partial S$ can be characterised by the vector field $\gamma'(\theta)$, which is taken to be single-valued. Throughout, we use the convention that the magnitude of a vector is indicated by omitting the overarrow, i.e., $\gamma'$. The “line element” $dl$ [cf. Eq. (13)] is then given by

$$dl = \left[ \gamma^2 + \left( \frac{d\gamma}{d\theta} \right)^2 \right]^{1/2} d\theta.$$  

Similarly, the normalised vectors tangent to $\partial S$ are given by

$$\hat{T} = \frac{1}{\sqrt{\gamma^2 + (d\gamma/d\theta)^2}} \left( \frac{d\gamma}{d\theta}, 1 \right)^T.$$  

Here, since the Christoffel symbols $\Gamma^l_{jk}$ are only non-zero in three cases: $\Gamma^1_{22} = -r$, and $\Gamma^2_{12} = \Gamma^2_{21} = 1/r$, the components of the covariant derivative $\nabla_j \hat{T}$ are

$$\left( \nabla_j \hat{T} \right)^1 = -\frac{\gamma \left[ \gamma^2 + 2(\gamma')^2 - \gamma \gamma'' \right]}{\left[ \gamma^2 + (\gamma')^2 \right]^2}, \quad \text{and} \quad \left( \nabla_j \hat{T} \right)^2 = \frac{\gamma' \left[ \gamma^2 + 2(\gamma')^2 - \gamma \gamma'' \right]}{\gamma \left[ \gamma^2 + (\gamma')^2 \right]^2},$$

where the shorthand $\gamma' = d\gamma/d\theta$ has been introduced for readability. Returning to (28), we see that

$$q_g = \frac{\gamma^2 + 2(\gamma')^2 - \gamma \gamma''}{\left[ \gamma^2 + (\gamma')^2 \right]^{3/2}}.$$  

If the membrane boundary is quasi-circular, then $\gamma(\theta) = R_0 \left[ 1 + \epsilon f(\theta) \right]$, where $f(\theta) = \sum_n \Re \{ A_n \exp(i n \theta) \}$, such that $\Re$ is the real part, $A_n \in \mathbb{C}$, $|A_n| \leq 1 \forall n \in \mathbb{N}$, and $\epsilon \ll 1$. A power series expansion in $\epsilon$ can then be performed on Eqs. (41) and (44), with the results

$$dl = R_0 \left\{ 1 + \epsilon \sum_{n} \Re \{ A_n \exp(i n \theta) \} + \frac{\epsilon^2}{2} \sum_{n} \Re \{ i A_n \exp(i n \theta) \}^2 \right\} d\theta + O(\epsilon^3),$$

and

$$q_g = \frac{1}{R_0} \left\{ 1 + \epsilon \sum_{n} (n^2 - 1) \Re \{ \exp(i n \theta) \} - \frac{\epsilon}{4} \sum_{n} \left\{ |A_n|^2 (3n^2 - 2) + \Re \{ A_n^2 \exp(2i n \theta) \} \right\} \right\} + O(\epsilon^3).$$

The energy $\mathcal{H}$ can then be shown to be of the form

$$\mathcal{H} = \pi \left[ k_g \left( \frac{q_0 R_0 - 1}{R_0} \right)^2 + R_0 (R_0 \sigma + 2 \tau) \right] + \epsilon^2 \frac{\pi}{4R_0} \left\{ k_g \left[ 2 + (q_0^2 R_0^2 - 5) n^2 + 2 n^4 \right] + 2 R_0^2 (R_0 \sigma + n^2 \tau) \right\} \sum_n |A_n|^2 + O(\epsilon^3).$$

The minimum of $\mathcal{H}$ is given by requiring $\partial \mathcal{H}/\partial R_0|_{\epsilon = 0} = 0$. The result is that

$$\tau = \frac{k_g}{2 R_0^2} (1 - q_0^2 R_0^2) - R_0 \sigma,$$  

Flat
Moreover, where \( \theta \) is the angle by which the angle brackets to indicate expectation value, this implies that each mode in equilibrium.

\[
\mathcal{H}_n = \frac{\pi c^2}{2 R_0} (n^2 - 1) [k_g (n^2 - 1) - R_0^3 \sigma] |A_n|^2 + O (\epsilon^3) .
\]

Equation (3) in the main text then follows from the above.

**Non-flat**

Parameterise the membrane shape by using the two (orthogonal) principal curvatures, chosen without loss of generality such that \(|c_1| \geq |c_2|\). The height field in an polar Monge approach is given by

\[
h(r,\theta) = \frac{r^2}{4} \left[ c_1 + c_2 + (c_1 - c_2) \cos 2\theta \right] ,
\]

where the angle \( \theta \) is assumed to be measured from the principal axis associated with \( c_1 \). Moreover, \( \| \nabla h \| \ll 1 \ \forall \ (r,\theta) \), therefore

\[
\max \{ \| \nabla h \| : \theta \in [0,2\pi], r \in [0,\gamma(\theta)] \} = \| \nabla h \|_{\theta=0,r=\gamma(0)} = \gamma(0) c_1 \ll 1 \quad (52)
\]

Since \( \gamma(\theta) = R_0 [1 + \epsilon f(\theta)] \), we formally set \( \alpha = R_0 c_1 \ll 1 \), therefore (51) becomes

\[
h(r,\theta) = \alpha \psi(r,\theta) , \quad \text{with} \ \psi(r,\theta) = \frac{r^2}{4 R_0} \left[ 1 + \phi + (1 - \phi) \cos 2\theta \right] , \quad (53)
\]

where \( \phi = c_2/c_1 \) takes values in the interval \([-1, 1]\). Making contact with Equation (49) \( S \) is parameterised by \( \{ u^\alpha : \alpha = 1, 2 \} \) and \( \mathbb{R}^3 \) by \( \{ x^i : i = 1, 2, 3 \} \), where a Monge gauge is tantamount to choosing the map \( F(u) = (u^1, u^2, h(u^1, u^2))^T \). Moreover, \( u^1 = r \) and \( u^2 = \theta \), which implies that the embedding basis \( \{ e_i : i = 1, 2, 3 \} \) is just that of cylindrical polars \( \{ \hat{r}, \hat{r}, \hat{\theta}, \hat{z} \} \). The position vector is then \( \mathbf{R}(r,\theta) := r \hat{r} + \alpha \psi(r,\theta) \hat{z} \), and tangent vectors to the surface are spanned by the set:

\[
\mathbf{F}_s (\hat{e}_1) = \frac{\partial \mathbf{R}}{\partial r} = \hat{r} + \alpha \frac{\partial \psi}{\partial r} \hat{z} , \quad \text{and} \quad \mathbf{F}_s (\hat{e}_2) = \frac{\partial \mathbf{R}}{\partial \theta} = r \hat{\theta} + \alpha \frac{\partial \psi}{\partial \theta} \hat{z} . \quad (54)
\]

Recalling the shorthand \( \psi_\alpha := \partial \psi / \partial u^\alpha \), the metric and its inverse become

\[
g_{\alpha\beta} = (\hat{e}_\alpha,\hat{e}_\beta) = \mathbf{F}_s (\hat{e}_\alpha) \cdot \mathbf{F}_s (\hat{e}_\beta) = \begin{pmatrix}
1 + \alpha^2 (\psi_1)^2 & \alpha^2 \psi_1 \psi_2 \\
\alpha^2 \psi_1 \psi_2 & r^2 + \alpha^2 (\psi_2)^2
\end{pmatrix} , \quad (55)
\]

and

\[
g^{\alpha\beta} = (g_{\alpha\beta})^{-1} = \frac{1}{r^2 (1 + \alpha^2 \| \nabla R^2 \psi \|^2) \left( r^2 + \alpha^2 (\psi_2)^2 \right)} \begin{pmatrix}
-\alpha^2 \psi_1 \psi_2 & -\alpha^2 \psi_1 \psi_2 \\
\alpha^2 \psi_1 \psi_2 & -\alpha^2 \psi_1 \psi_2
\end{pmatrix} . \quad (56)
\]

It is clear that \( g := \text{Det}(g_{\alpha\beta}) = r^2 \left( 1 + \| \nabla R^2 \psi \|^2 \right) \), where \( \nabla R^2 \) is the covariant derivative in \( \mathbb{R}^2 \) (polar coordinates are assumed). We may also calculate the normal to the surface [cf. Eq. (12)],

\[
n = \hat{z} \left( 1 - \alpha^2 \| \nabla R^2 \psi \|^2 \right) - \hat{r} \alpha \psi_1 - \hat{\theta} \alpha \psi_2 + O (\alpha^3) , \quad (57)
\]
and the derivatives of the tangent vectors (pushed forwards to $\mathbb{R}^3$), i.e.,

$$\frac{\partial F_\alpha (\bar{e}_1)}{\partial u^1} = \frac{\partial^2 R}{\partial r^2} = \alpha \psi_{,11} \hat{z}, \quad \frac{\partial F_\alpha (\bar{e}_2)}{\partial u^2} = \frac{\partial^2 R}{\partial \theta^2} = -r \hat{r} + \alpha \psi_{,22} \hat{z}, \quad \text{and} \quad \frac{\partial F_\alpha (\bar{e}_1)}{\partial u^1} = \frac{\partial^2 R}{\partial r \partial \theta} = \hat{\theta} + \alpha \psi_{,12} \hat{z},$$

from which the coefficients of the second fundamental form $[24]$ may be constructed:

$$b_{\alpha \beta} = \alpha \left( \psi_{,11} \psi_{,12} - \psi_{,12} \psi_{,22} - \frac{\psi_{,22}}{r} \psi_{,11} + \frac{\psi_1}{r^2} \right) + O (\alpha^3).$$

Using the definitions $[33]$ and $[36]$ it can be shown that, up to second order in the small parameter $\alpha$, the mean and Gaussian curvatures are just the trace and determinant of the (polar coordinate) Hessian of $\psi$. That is,

$$H = \text{Tr} \left[ \alpha \text{Hess}^{\mathbb{R}^2} (\psi) \right] + O (\alpha^3) = \alpha \frac{\alpha^2}{r^2} \left( \psi_{,11} + \frac{\psi_{,12}}{r} + \frac{\psi_{,22}}{r^2} \right) + O (\alpha^3),$$

and

$$K = \frac{1}{r^2} \text{Det} \left[ \alpha \text{Hess}^{\mathbb{R}^2} (\psi) \right] + O (\alpha^3) = \alpha \frac{\alpha^2}{r^2} \left[ \psi_{,11} (r \psi_{,1} + \psi_{,22}) - \left( \psi_{,12} - \frac{\psi_2}{r} \right)^2 \right] + O (\alpha^3),$$

where

$$\left[ \text{Hess}^{\mathbb{R}^2} (\psi) \right]_{\alpha \beta} := \psi_{,\alpha \beta} = \psi_{,\alpha \beta} - \mathbb{R}^2 \Gamma_{\alpha \beta}^{\gamma} \psi_{,\gamma}.$$

Substituting for $[53]$ leads to the results:

$$H = \frac{\alpha}{R_0} (1 + \phi) + O (\alpha^3), \quad \text{and} \quad K = \frac{\phi \alpha^2}{R_0^2} + O (\alpha^3).$$

That is, up to $O (\alpha^2)$, the mean and Gaussian curvatures of a perturbation of the form $[53]$ are constant.

In a similar way to the above treatment of $H$ and $K$, both the line element $dl$, and curvatures $q_g$ and $q_n$ may be expanded in terms of $\alpha$. However, these quantities also rely on $\epsilon$. To avoid confusion between power series expansions of $\alpha$ and $\epsilon$, we adopt the notation that coefficients are labelled in the following way: in an expansion of some function $F$, the $O (\alpha^a \epsilon^b)$ term is written as $\alpha^a \epsilon^b F^{(\alpha, \epsilon)}$. For example, using this convention, the results of the previous section can be re-labelled. The terms of $[45]$ become

$$dl^{(0,0)} = R_0 d\theta, \quad dl^{(0,1)} = R_0 \sum_{n} \Re \left[ A_n \exp (i n \theta) \right] d\theta, \quad \text{and} \quad dl^{(0,2)} = \frac{R_0}{2} \sum_{n} n \Re \left[ i A_n \exp (i n \theta) \right]^2 d\theta,$$

while the terms of $[46]$ are given by

$$q_g^{(0,0)} = \frac{1}{R_0}, \quad q_g^{(0,1)} = \frac{1}{R_0} \sum_{n} (n^2 - 1) \Re \left[ \exp (i n \theta) \right],$$

and

$$q_g^{(0,2)} = \frac{-1}{4 R_0} \sum_{n} \left\{ |A_n|^2 (3n^2 - 2) + \Re \left[ A_n^2 \exp (2i n \theta) \right] \right\}.$$  

The full expansions, now in terms of both $\alpha$ and $\epsilon$, take the form

$$dl = dl^{(0,0)} + \epsilon dl^{(0,1)} + \epsilon^2 dl^{(0,2)} + \alpha^2 dl^{(2,0)} + O (\epsilon^3) + O (\alpha^3),$$

and

$$q_g = q_g^{(0,0)} + \epsilon q_g^{(0,1)} + \epsilon^2 q_g^{(0,2)} + \alpha^2 q_g^{(2,0)} + O (\epsilon^3) + O (\alpha^3),$$

where

$$dl^{(1,0)} = 0, \quad \text{and} \quad dl^{(2,0)} = \frac{\psi_{,2} (R_0, \theta)^2}{2 R_0} = \frac{R_0}{8} (\phi - 1)^2 \sin^2 (2\theta),$$
and
\[ q_\gamma^{(1,0)} = 0, \quad \text{and} \quad q_\gamma^{(2,0)} = \frac{1}{2 R_0^2} \left[ -2 \psi_{,2}(R_0, \theta)^2 + \psi_{,22}(R_0, \theta)^2 \right] = \frac{1}{8 R_0^2} (1 - \phi)^2 \left[ 1 + 3 \cos(4 \theta) \right]. \] (70)

For the normal curvature, we have
\[ q_n = \alpha q_n^{(1,0)} + O \left( \alpha^3 \right) + O \left( \epsilon^3 \right), \]
where
\[ q_n^{(1,0)} = \frac{1}{R_0^2} \left[ \psi_{,22}(R_0, \theta) + R_0 \psi_{,1}(R_0, \theta) \right] = \frac{1}{2 R_0^2} \left[ 1 + \phi + (\phi - 1) \cos(2 \theta) \right]. \] (71)

Substituting the above results into Eq. (49), the necessary integrals can be performed in order to obtain an expansion of \( \mathcal{H} \) in terms of both \( \alpha \) and \( \epsilon \). (Note: the manipulations are quite tedious and we used the commercial software Mathematica [25]). If the spontaneous (mean) curvature \( c_0 \) is nonzero, then the expansion contains a term
\[ \mathcal{H}^{(1,0)} = -2 c_0 \kappa \pi R_0 (1 + \phi), \] (72)

In this case, the up / down symmetry of the problem is broken, and all perturbations of the form \( \mathcal{H}^{(1,0)} \), other than a symmetric saddle, are unstable. (A symmetric saddle is given by \( c_1 = c_2 \), such that \( \phi = -1 \) and \( H = 0 \)). By contrast, if the spontaneous curvature is zero, then the energy is of the form
\[ \mathcal{H} = \mathcal{H}^{(0,0)} + \epsilon^2 \mathcal{H}^{(0,2)} + \alpha^2 \mathcal{H}^{(2,0)} + O \left( \epsilon^3 \right) + O \left( \alpha^3 \right). \] (73)

Here, the line tension \( \tau \) can once again be fixed for a given \( R_0 \) (and material parameters \( k_g, k_n, q_0 \) and \( \sigma \)) by imposing \( \partial \mathcal{H} / \partial R_0 |_{\epsilon=\alpha=0} = 0 \). The result is unchanged from (48). In this case \( \mathcal{H}^{(0,0)} \) and \( \mathcal{H}^{(0,2)} \) can then be read-off from Eq. (49), i.e.,
\[ \mathcal{H}^{(0,0)} = \frac{\pi}{2} \left[ k_g \left( 1 - q_0 R_0 \right) - \frac{R_0^2 \sigma}{2} \right], \quad \text{and} \quad \mathcal{H}^{(0,2)} = \frac{\pi}{2 R_0} (n^2 - 1) \left[ k_g \left( n^2 - 1 \right) - R_0^2 \sigma \right] \sum_n |A_n|^2. \] (74)

The coefficient of \( \alpha^2 \) in (73) is given by
\[ \mathcal{H}^{(2,0)} = \pi \left\{ (1 + \phi)^2 \frac{2}{k_g} + \left[ (1 + \phi)^2 + 2 \left( 1 + \phi^2 \right) \right] \frac{k_n}{8 R_0} + \phi \left( \frac{R_0^2 \sigma}{4} + q_0 k_g \right) \right\}. \] (75)

which can be re-written in the form \( \mathcal{H}^{(2,0)} = \pi \phi [\bar{\kappa} - \bar{\kappa}^*(\phi)] \), where
\[ \bar{\kappa}^* = -\frac{1}{\phi} (1 + \phi)^2 \frac{3 k_g}{R_0^2} - \frac{R_0^2 \sigma}{4} - \frac{1}{\phi} \left[ (1 + \phi)^2 + 2 \left( 1 + \phi^2 \right) \right] \frac{k_n}{8 R_0} - q_0 k_g, \] (76)
determines the stability of out-of-plane perturbations. Setting \( \phi = -1 \) recovers
\[ \bar{\kappa}^*_p = \bar{\kappa}^*(-1) = \left( \frac{3}{2 R_0} - q_0 \right) k_g + \frac{k_n}{2 R_0} - \frac{R_0^2 \sigma}{4}, \] (77)
as shown in the main text.

**Higher order contributions**

Consider the effect of contributions to the energy of order greater than \( \alpha^2 \). In the expansion of \( \mathcal{H} \), the terms of next lowest order can be calculated, and are at order \( \alpha^4 \). However, to this order, \( \epsilon \) and \( \alpha \) do not de-couple, and we must explicitly set \( \epsilon = 0 \). [In doing so, we revert to the simple notation that \( O(\alpha^n) \) terms in a series expansion of a given function, say \( F \), are written \( \alpha^n F^{(n)} \)]. In addition, when expanding to \( O(\alpha^4) \), the Helfrich Hamiltonian must be modified since Eq. (37) retains only lowest order terms (\( \alpha^2 \)) by construction. In our framework—i.e., shapes
described by the polar monge field \( \tilde{\phi} \) — the requisite higher order terms can be computed, and are given by

\[
H^4 = \frac{\alpha^4}{16 R_0^4} (1 + \phi)^4 + O (\alpha^5)
\]

(78)

\[
H^2 K = \frac{\alpha^4}{4 R_0^4} \phi (1 + \phi)^2 + O (\alpha^5),
\]

(79)

\[
K^2 = \frac{\alpha^4}{R_0^4} \phi^2 + O (\alpha^5),
\]

(80)

\[
\Delta H^2 = -\frac{\alpha^4}{2 R_0^4} (1 + \phi)^2 [3 (1 + \phi^2) - 2 \phi] + O (\alpha^5),
\]

(81)

\[
\Delta K = -\frac{4 \alpha^4}{R_0^4} \phi (1 + \phi^2) + O (\alpha^5).
\]

(82)

When multiplied by their respective moduli and integrated, these terms appear alongside other \( O(\alpha^4) \) terms, which arise from the expansions of (37) and (38). Assigning the coefficients \( \lambda_i \) (for \( i = 1 \ldots 5 \)) to the contributions (78) to (82), respectively, the resultant fourth order contribution to the energy \( \mathcal{H}^{(4,0)} \) is given by

\[
\mathcal{H}^{(4,0)} = -\frac{3\pi}{8} \phi (1 + \phi^2) \left[ \kappa - \kappa_p \right],
\]

(83)

where

\[
\kappa = \frac{8}{3 \phi (1 + \phi^2)} \left\{ \kappa \left[ \frac{\alpha}{4} - \frac{3 \phi^2 - \phi - 2 \phi^3}{2} - \frac{5 \phi^4}{4} \right] + \frac{k_g}{R_0} \left[ \frac{19}{512} - \left( \frac{3 \phi_0 R_0}{8} - \frac{19}{128} \right) \phi - \frac{57 \phi^2}{256} - \left( \frac{3 \phi_0 R_0}{8} - \frac{19}{128} \right) \phi^3 \right] \right. \\
+ \frac{\sigma R_0^2}{k_g} \left[ \frac{5}{512} - \frac{3 \phi}{128} + \frac{19 \phi^2}{768} - \frac{3 \phi^3}{128} + \frac{5 \phi^4}{512} \right] + \frac{\lambda_3}{R_0^2} \phi^2 - \frac{\alpha}{4} \left( \frac{3}{2} (1 + \phi^2) - \phi \right) \left\} \right.
\]

(84)

Focusing, as before, on saddles with principal curvatures of equal magnitude (so-called “pringles”) we set \( \phi = -1 \), resulting in

\[
\kappa = \frac{19 k_g}{24 R_0} + \frac{11 k_n}{12 R_0} - k_g q_0 - \frac{5}{72} R_0^2 \sigma - \frac{4}{3} \frac{\lambda_3}{R_0^2} - \frac{32}{3} \frac{\lambda_5}{R_0^2},
\]

(85)

where only \( \Delta K \) and \( K^2 \) (i.e., the terms involving Gaussian curvature) contribute from the higher order modifications (78) to (82). The coefficients in the expansion of the energy are now given by \( \mathcal{H}^{(2)} = -\pi (\kappa - \kappa_p) \) and \( \mathcal{H}^{(4)} = 3 \pi (\kappa - \kappa_p)/4 \). Due to the introduction of order \( \alpha^4 \) contributions, there are now solutions to \( \partial \mathcal{H}/\partial \alpha = 0 \) at non-zero \( \alpha \), given by

\[
\alpha^* = \pm \left( \frac{-\mathcal{H}^{(2)}}{2 \mathcal{H}^{(4)}} \right)^{-1/2}.
\]

(86)

The stability is determined by the coefficient \( \mathcal{H}^{(2)} \), via

\[
\frac{\partial^2 \mathcal{H}}{\partial \alpha^2} \bigg|_{\alpha = \alpha^*} = -4 \mathcal{H}^{(2)},
\]

(87)

which implies that \( \alpha^* \) is only stable if \( \kappa > \kappa^*_p \). In addition, from (86), the expression \( -\mathcal{H}^{(2)}/2 \mathcal{H}^{(4)} \) must be positive, and we can therefore deduce that \( \kappa > \max (\kappa^*_p, \kappa^*_p) \) for stable pringles.
Consider a two component nanodiscoid, where the each component has a different modulus of Gaussian rigidity, $\bar{\kappa}_1$ and $\bar{\kappa}_2$. If $\mu$ is an area fraction, we write $\bar{\kappa} = \mu \bar{\kappa}_1 + (1 - \mu) \bar{\kappa}_2$ as the average modulus of Gaussian rigidity of the (well mixed) membrane from which the discoids are cut. Relative to the bulk membrane, a pringle on the cusp of formation may contain an additional area fraction $\psi$ of one of the components, resulting in a Gaussian rigidity for the pringle of the form $\bar{\kappa} + \psi \delta \bar{\kappa}$, with $\delta \bar{\kappa} = \bar{\kappa}_1 - \bar{\kappa}_2 > 0$ the difference between the Gaussian rigidities of each component. The membrane Hamiltonian might then include the extra term

$$H_\psi = \int_S dA \left( \frac{\chi \psi^2}{2} \right),$$

with $\chi$ a Flory-like parameter that approaches zero the bulk membrane approaches the demixing transition.

For nanodiscoids subject to a pringle shaped ($\phi = -1$) perturbation,

$$H_\psi = \frac{\chi \psi^2 \pi R_0^2}{2} \left( 1 + \frac{\alpha^2}{4} - \frac{\alpha^4}{48} \right) + O(\alpha^5).$$

The total energy $H = H_m + H_b + H_\psi$ is then

$$H = \pi \left\{ \left[ \frac{k_g}{R_0} (1 - q_0 R_0) - \frac{R_0^2 \sigma}{2} + \frac{\chi \psi^2 R_0^2}{2} \right] + \alpha^2 \left[ \frac{\chi \psi^2 R_0^2}{8} - (\bar{\kappa} + \psi \delta \bar{\kappa} - \bar{\kappa}_p) \right] \right. \right.$$  

$$+ \alpha^4 \left[ \frac{3}{4} (\bar{\kappa} + \psi \delta \bar{\kappa} - \bar{\kappa}_p^\dagger) - \frac{\chi \psi^2 R_0^2}{96} \right] \right\} + O(\alpha^5).$$

The additional area fraction $\psi$ of the pringle’s surface that is occupied by the component $\bar{\kappa}_1$ is found by imposing $\partial H / \partial \psi = 0$, which implies

$$\psi^2 = \frac{(\delta \bar{\kappa})^2}{\chi^2 R_0^4} \alpha^4 + O(\alpha^5).$$

Substituting into (89) gives

$$H_\psi = \frac{\pi (\delta \bar{\kappa})^2}{2 \chi R_0^2} + O(\alpha^5),$$

and hence

$$H = \pi \left\{ \left[ \frac{k_g}{R_0} (1 - q_0 R_0) - \frac{R_0^2 \sigma}{2} \right] - \alpha^2 (\bar{\kappa} - \bar{\kappa}_p^*) + \frac{3 \alpha^4}{4} \left[ \bar{\kappa} - \left( \bar{\kappa}_p^* - \frac{2 (\delta \bar{\kappa})^2}{3 \chi R_0^2} \right) \right] \right\} + O(\alpha^5).$$

Writing

$$\bar{\kappa}_p^\dagger = \bar{\kappa}_p^\dagger - \frac{2 (\delta \bar{\kappa})^2}{3 \chi R_0^2},$$

implies non-zero stable solutions $\alpha^*$, and hence principal curvatures of magnitude

$$|c_1| = \frac{1}{R_0} \left[ \frac{2 (\bar{\kappa} - \bar{\kappa}_p^*)}{3 (\bar{\kappa} - \bar{\kappa}_p^\dagger)} \right]^{1/2},$$

for average Gaussian curvatures $\bar{\kappa} > \max (\bar{\kappa}_p^*, \bar{\kappa}_p^\dagger)$.

**IMAGE ANALYSIS**

Two cryo Electron Microscopy images were analysed, one is shown in Fig. 3 of the main manuscript, and the other is displayed in Fig. Images were analysed according to the following protocol. In order to remove unwanted noise,
images were pre-processed using the free application ImageJ. After conversion to an 8-bit single channel image, a small Gaussian blur was applied (10 pixel variance) followed by a low pass filter, set to remove structures below 15 pixels in size (1 pixel = 0.131 nm). The identification and fitting of shapes was carried out using the freely available OpenCV library of Python functions. First, the pre-processed image was thresholded, after which discoid contours were extracted. Ellipses were then fitted to the discoid contours using an in-built least-squares procedure. We remark that the protocol relies on edge-detection via contrast, and hence is not well suited to differentiating between overlapping SMALPs or identifying those that are out of the focal plane.

In total, the analysis identified 414 SMALPs across both images. The average length of the ellipse semi-major axes was 3.48 nm, with a variance of 1.68 nm$^2$. 