

Aggregates of Two-Dimensional Vesicles: Rouleaux, Sheets, and Convergent Extension

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Using both numerical and variational minimization of the bending and adhesion energy of two-dimensional lipid vesicles, we study their aggregation, and we find that the stable aggregates include an infinite number of vesicles and that they arrange either in a columnar or in a sheetlike structure. We calculate the stability diagram and we show that the sheetlike aggregate can be transformed into the columnar aggregate via vesicle intercalation, which makes the transformation reminiscent of the process of convergent extension observed in some biological tissues.

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Since Hooke's *Micrographia*, shapes of aggregates of simple cells have fascinated biologists, physicists, and mathematicians alike. In the part of Thompson's classic *On Growth And Form* devoted to the "forms of cells" and "cell-aggregates" [1], the main concept used to interpret the regularity of many aggregates is surface tension which forces cells to minimal-area configurations. Of course, Thompson viewed surface tension merely as a mesoscopic manifestation of the complex biochemical apparatus and the structure of a cell. Similar ideas are still being explored: Recently, cells of the developing *Drosophila* retina were shown to pack just like soap bubbles [2]. Another concept related to some cells (e.g., erythrocytes [3]) is that of a vesicle formed by a closed lipid bilayer membrane [4]. The structure of a vesicle aggregate held together by intermembrane attraction is determined by both adhesion and membrane elastic energy rather than by surface energy alone as in a soap froth.

Regardless of their mechanical framework—soap bubble [2], lipid vesicle [4], or a Potts Hamiltonian [5]—phenomenological models of cell aggregates are most transparent within the context of undifferentiated systems with distinct geometry, e.g., early embryonic stages or layered tissues of epithelial sheets. Some epithelia consist of a single layer of prismatic cells and their salient geometrical feature is their cross-section, essentially a polygonal partition of a plane. In many cases, the statistics of two-dimensional (2D) partitions obey simple empirical relationships such as the Lewis law first observed in cucumber epidermis [6] and the Aboav-Weaire law [7]. In a soap-froth-like partition, the link between its structure and the local equilibrium is embodied in the Plateau rules, one of the premises used to clarify the above laws [8,9]. On the other hand, no such rules exist for partitions formed by vesicle aggregates: Their energy functional is more complicated than that of a soap froth, and their minimal-energy configuration is unknown.

In this Letter, we study aggregates of 2D vesicles as a model which could elucidate some structural aspects of simple layered tissues. By employing the elasticity theory

combined with the contact-potential intermembrane attraction, we build on existing insight into free 2D vesicles [10] and their adhesion on flat substrates [11]. We focus on vesicles of identical area and perimeter, and we find that the stable aggregates consist of infinitely many vesicles. We analyze both columnar and sheetlike aggregates, discuss the modes of transformation between them, and construct the stability diagram.

Our model is based on the 2D version of the lipid bilayer bending energy $W_b = (K/2) \oint C^2(s) ds$, where K is the bending constant and C is the local curvature [12]. The integral is evaluated along the contour of a vesicle with a fixed perimeter L and a fixed enclosed area A . The characteristic length scale is given by R_c , the radius of a circle of perimeter L , such that the reduced vesicle perimeter is normalized to $\oint ds/2\pi R_c = 1$ and the reduced area $a = \int dA/\pi R_c^2$ ranges between 0 and 1. The aggregate energy includes the bending energies of all members and the adhesion energy proportional to the total length of the contact lines: $W_a = -\Gamma \sum_i \int_{\text{contact}} ds_i$ [13]. Here Γ is the adhesion strength; the sum runs over all contact lines and the integral over their contours. The energy scale is given by $\pi K/R_c$, the bending energy of the reference vesicle with $a = 1$, and the reduced adhesion strength is $\gamma = \Gamma R_c^2/2K$ [14].

By minimizing the energy numerically using SURFACE EVOLVER [15], we first reproduce the free vesicle shapes [10]. For $a = 1$, the vesicle is a circle, for $0.8 \leq a < 1$ it adopts an ellipsoidal shape, for $0.27 < a \leq 0.8$ it is characterized by two invaginations on its long sides, and for $a < 0.27$ it consists of two tether-connected buds (Fig. 1). Their elongated shape determines the way vesicles preferentially stick to each other—the contact line of a pair is longest if the vesicles' long axes are parallel.

This is confirmed by the doublet shapes; a typical example is shown in Fig. 1. The doublets show that a column with 1D vesicle connectivity (briefly called a *rouleau* due to its topological equivalence to the characteristic red blood cell aggregate) is a good aggregate candidate. We analyzed its stability as both number of members and

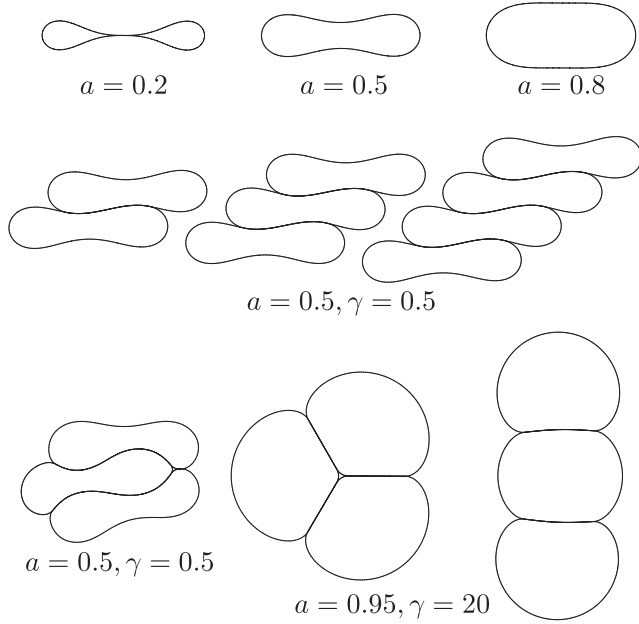


FIG. 1. Representative numerically obtained vesicle and aggregate shapes. Top: Free vesicles of small ($a = 0.2$), intermediate ($a = 0.5$), and large reduced areas ($a = 0.8$). Center: Doublet, columnar triplet, and columnar quadruplet for $a = 0.5$ and reduced adhesion strength $\gamma = 0.5$. Bottom: The energy of a trefoil exceeds that of a columnar triplet unless a and γ are large. The $a = 0.5$, $\gamma = 0.5$ trefoil is metastable, its $a = 0.95$, $\gamma = 20$ counterpart is stable. Also shown is the metastable columnar triplet for $a = 0.95$ and $\gamma = 20$.

adhesion strength are varied, finding that the threshold for rouleau formation decreases with the number of members. For example, the columnar aggregates of 2, 3, and 4 vesicles with $a = 0.5$ are stable for reduced adhesion strengths beyond 0.061, 0.052, and 0.048, respectively; the extrapolated aggregation threshold for the infinite rouleau is at $\gamma = 0.042$. Thus the stable rouleau consists of infinitely many vesicles, which is plausible: The more members in an aggregate, the longer the average contact line per vesicle and the lower the threshold.

The rouleau is not the only possible arrangement of vesicles but our numerical studies of other topologies of triplets and quadruplets at various a and γ strongly indicate that these are relevant exclusively in finite aggregates above a certain adhesion strength which increases dramatically as the vesicle area is decreased. As an illustration, we note that even in vesicles of area as large as 0.95, the trefoil is stable compared to the rouleau triplet only for γ beyond 10.9 which is well above the aggregation threshold ≈ 0.5 as shown below. But the total contact line of the middle vesicle in the $a = 0.95$ rouleau triplet is longer than the average total contact line per vesicle in the trefoil regardless of γ , and thus the energy of a rouleau with a large enough number of members is always lower than that of a set of trefoils. This supports the conclusion that the stable rouleau is infinite.

Figure 1 shows that the contact lines of vesicles in an infinite rouleau can be either sigmoidal or flat [16]. This is exemplified by the central pair of vesicles in the numerically obtained ten-member $a = 0.2$ rouleaux (Fig. 2). The outer contact lines of the pair are almost parallel both at $\gamma = 25$ where they are sigmoidal and at $\gamma = 40$ where they are almost flat, which means that the vesicle pair may serve as the repeat unit of an infinite stack.

Currently, a complete numerical analysis of vesicle aggregates is well beyond reach even with advanced tools such as SURFACE EVOLVER. Instead, we use the above clues to construct a variational model of an infinite rouleau. We describe the sigmoid-contact vesicle by two circular caps that together make up a full circle and two identical one-wave sinusoids combined into a smooth contour (Fig. 3) which can be done such that the phases of the sinusoids are either identical or opposite giving *S*- and pear-shaped vesicles, respectively. The model flat-contact vesicle consists of two identical straight lines connected by half-ellipses. Using the fixed-area and fixed-perimeter constraints, two out of the three parameters of the model shapes can be expressed in terms of the remaining one by resorting to analytical approximations of the elliptic integral, and the value of the free parameter is then varied so as to minimize the total energy.

This model reproduces the continuous transition from the sigmoid-contact repeat unit (with a staircase-like or a zigzag stacking of vesicles) to the flat-contact repeat unit (which gives straight aggregates) implied by the numerically calculated shapes of the ten-member rouleau for γ between 25 and 40. As γ is increased beyond the transition, the model flat-contact rouleau undergoes a telling transformation: The length of the contact line and the eccentricity of the vesicle cap both grow such that its shape approaches a rounded rectangle (Fig. 3). This suggests that at large γ , a sheetlike vesicle aggregate with 2D connectivity may be preferred to a rouleau with 1D connectivity (or several disconnected rouleaux). To explore this possibility, we construct a model sheetlike aggregate using convex vesicles with a contour consisting of straight contact lines and circular arcs of identical radii (Fig. 3). We find that the reduced energy of this vesicle is $\sqrt{2\gamma}(2 - \sqrt{2\gamma})$ independent of a , number of sides, and relative sizes of the straight sections and the internal angles; the reduced

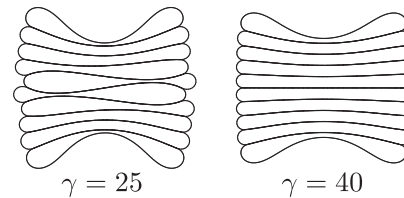


FIG. 2. Rouleaux of ten $a = 0.2$ vesicles for $\gamma = 25$ and 40. In both cases, the outer contact lines of the central pair are nearly parallel, showing that the pairs can serve as sigmoid-contact—flat-contact repeat units of an infinite rouleau.

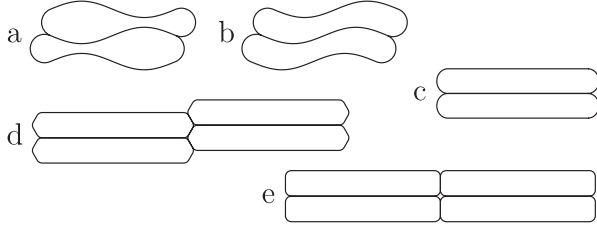


FIG. 3. Model $a = 0.4$ vesicles in sigmoid-contact rouleau [(a) pear-shaped, (b) S -shaped version; $\gamma = 1$] and in flat-contact rouleau [(c) $\gamma = 19$]. Also shown are examples of model vesicles in sheetlike aggregate introduced in the text [(d) hexagons, (e) rectangles; $\gamma = 45$]. Regular tilings are special cases of the sheet geometry, which is generally disordered as argued in the text.

equilibrium curvature of the rounded corners is $\sqrt{2\gamma}$ in agreement with the boundary condition at the edge of the contact zone [17].

Using all model aggregates, we now delineate their stability diagram (Fig. 4); the aggregation threshold is outlined by comparing them to the numerically calculated free vesicle shapes. Indeed the sequence observed upon increasing adhesion strength is free vesicle, rouleau, or sheet unless the reduced vesicle area is too small or too large; for $a < 0.45$, the rouleau region is subdivided into the sigmoid contact and the flat-contact part. For $a < 0.27$ where a free vesicle consists of two tether-connected buds,

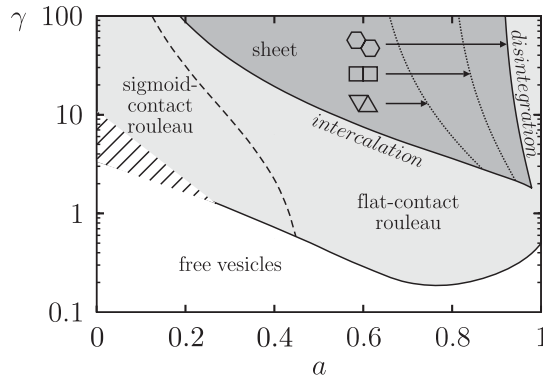


FIG. 4. Stability map of adhering 2D vesicles is divided into regions of free vesicles, infinite rouleaux, and infinite sheetlike aggregates. The sigmoid-contact–flat-contact rouleau transition is shown by the dashed line; the large-area limit of rouleau stability is infinitesimally smaller than 1 as at $a = 1$ the vesicles can only be free. The largest reduced areas supporting periodic sheet tilings with three, four, and sixfold coordination correspond to the two dotted lines and the right-hand boundary of the sheet region, each labeled by the respective schematic repeat unit. Also indicated are the modes of the sheet-rouleau transition described in the text. For $a < 0.27$, free vesicles reduce to two tether-connected buds, and in the parameter space qualitatively outlined by the crossed region they form aggregates more complex than those discussed here.

the small- γ rouleaux are more complicated than those studied here; we did not explore this regime in detail.

Since the rounded polygonal model vesicles are energy degenerate, the sheetlike aggregate should comprise coexisting triangles, tetragons, pentagons, hexagons, etc., conceivably locally aligned. Such coexistence is typical of the most part of the sheet region. But as a increases the set of allowed shapes is gradually narrowed and the sheet becomes more ordered, which is illustrated by the maximal reduced vesicle areas where a periodic tiling with three, four, and sixfold coordination is possible at a given γ (Fig. 4); the largest a which supports a sheet corresponds to a rounded regular hexagon. A potentially relevant feature of the sheet is the fraction of the plane not covered by the vesicles (Z). Z can be evaluated easily for regular tilings: To lowest order, $Z = \beta/\gamma a$ where β equals $(2\sqrt{3} - \pi)/2\pi \approx 0.051$ for a tiling of equiangular hexagons and $(4 - \pi)/2\pi \approx 0.137$ for a rectangular tiling. Z is typically small; for equiangular hexagons, its upper limit reached at $a = 0.98$ and $\gamma = 1.8$ is $Z = 2.9\%$.

On a quantitative note, the aggregation threshold is overestimated in our analysis. For example, the numerically obtained value for $a = 0.5$ vesicles is at $\gamma = 0.042$ rather than at $\gamma = 0.46$ as shown in Fig. 4. This is expected as the variational energies are too large but we estimate that for $\gamma \geq 0.5$ the discrepancy does not exceed 10%, and we stress that it decreases rapidly with γ . This is illustrated by the finite $\gamma = 25$ and 40 rouleaux (Fig. 2) whose central vesicles are described faithfully by the ansatz shapes (Fig. 3); the numerically calculated transition between the two types of the ten-member rouleau with $a = 0.2$ is at $\gamma \approx 32$ very close to $\gamma = 31$ as predicted variationally for the infinite rouleau. Thus the model works well at large γ and we trust that the sheet-rouleau boundary (Fig. 4) is quite accurate.

The transition between a 2D-connected sheet and a 1D-connected rouleau is a very interesting aspect of the system, and it can be accomplished either by disintegration of a sheet into several rouleaux or by vesicle intercalation transforming a sheet into a single rouleau. Disintegration is preferred along the right-hand boundary of the sheet region where the sheet consists of a periodic tiling of rounded regular hexagons which can break up into parallel rouleaux along a lattice axis. In contrast, at the left-hand boundary the sheet is disordered and cannot dissolve into separate rouleaux. But the vesicles can intercalate, thereby gradually decreasing the extent of a sheet along their average long axis. In our model, intercalation is facilitated by the degeneracy of the energy of vesicles so that they may change their shape at a given reduced area as appropriate at no cost.

These results may be related to convergent extension [18], a morphogenetic mechanism where cells in a layered tissue (i) undergo in-plane elongation, (ii) align, and (iii) intercalate such that the tissue is extended perpendicu-

lar to the long axes of cells. This phenomenon can be described in terms of the differential adhesion hypothesis by assuming that the en-face cross-section of cells is elongated, say rectangular with 2 long and 2 short sides, and that the adhesion strengths for long-long, long-short, and short-short contacts are all different [19]. Under certain conditions, this gives a minimal-energy aggregate that is orientationally ordered and elongated normal to cell axes, thus reproducing the final stage of convergent extension. But the simplest microscopic basis of differential adhesion would be a variation of the density of anchoring junctions across the cell sides, and there is no solid evidence either for or against this [19].

The stability diagram (Fig. 4) offers an alternative interpretation: Mechanically, convergent extension could be the process of sheet-rouleau transition induced by a decrease of adhesion strength or reduced area of the en-face cell cross-section, whereby the sheet would seek the energy minimum by transforming into a rouleau via intercalation. In real tissues, convergent extension is certainly not driven solely by the interplay of the intermembrane adhesion and cell elastic energy as a passive, nonspecific force; the sheet-rouleau transition may be either promoted or impeded by the action of cytoskeleton and cell structures as well as by the interaction of the cell with the extracellular matrix and the surrounding tissues. Nonetheless, our scenario is consistent with several features of the process unaccounted for by the differential adhesion model. For example, convergent extension can occur in the absence of changes in the cell shape [20] (in this case, it could be induced by a decrease of adhesion strength), and it can be accompanied by the onset of disorder where sixfold cell coordination is replaced by patterns including a range of polygonal shapes [21] (which happens in the sheetlike aggregate as the reduced vesicle area is decreased). Thus the differential adhesion model [19] can be regarded as an effective, coarse-grained version of our theory where the vesicle elastic energy is absorbed in an anisotropic adhesion constant.

In conclusion, our study of adhering 2D vesicles shows that the structure of minimal-energy aggregate is simplified by the fact that the stable aggregates, both columnar and sheetlike, are infinite. The main results should be qualitatively insensitive to a moderate vesicle polydispersity, and they call for a generalization to 3D vesicles.

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