VERTEX MODELS AND THREE-DIMENSIONAL
EPITHELIAL MORPHOGENESIS

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Abstract

Regulated deformations of epithelial sheets are frequently foreshadowed by patterning of their mechanical properties. The connection between patterns of cell properties and the emerging tissue deformations is studied in multiple experimental systems, but the general principles remain poorly understood. For instance, it is in general unclear what determines the direction in which the patterned sheet is going to bend and whether the resulting shape transformation will be discontinuous or smooth.

In chapter 2, these questions are explored computationally, using vertex models of epithelial shells assembled from prism-like cells. In response to rings and patches of apical cell contractility, model epithelia smoothly deform into invaginated or evaginated shapes similar to those observed in embryos and tissue organoids. Most of the observed effects can be captured by a simpler model with polygonal cells, modified to include the effects of the apicobasal polarity and natural curvature of epithelia. Our models can be readily extended to include the effects of multiple constraints and used to describe a wide range of morphogenetic processes.

In chapter 3, we employ the recently proposed modified vertex model to systematically explore the connection between the two-dimensional patterns of cell properties and the emerging three-dimensional structures. We illustrate it through the computational analysis of dorsal appendage morphogenesis in the developing Drosophila egg. We conclude this thesis by discussing possible future directions to extend this work.
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To my nani and parents.
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Chapter 1

Introduction

Epithelium is the first tissue to emerge during the development of an embryo and forms most of the biological structures [19]. Epithelial cells are characterized by tight attachments to each other forming a closely packed sheet (Fig. 1.1A,B). Epithelial monolayer shows apical-basal polarity and undergoes controlled deformations to transform into complex shapes. Such morphogenetic transformations can be divided into two major classes first, localized bending leading to tissue folding (Fig. 1.1C) and second, reshaping of epithelial sheet driven by cell rearrangements (Fig. 1.1D). These epithelial movements are triggered by spatial patterns of extracellular signals that remodel actomyosin, adherens junctions and other cytoskeletal components [60].

Some well-studied examples of epithelial morphogenesis that involve bending include blastula-to-gastrula transition [33, 65], neural plate invagination [28], optic [11], and otic vesicle formation. In these examples, the shape transformation involves localized invagination without cell rearrangements or increase in the number of cells. Similarly, there are multiple instances of shape transformations that are driven by cell rearrangements. Some examples are germband extension during Drosophila embryogenesis [31], epiboly during zebrafish embryogenesis [32], and dorsal appendage formation during Drosophila oogenesis [48, 8]. One can use computational models...
to understand the role of mechanics in such morphogenetic processes, test different hypothesis and provide predictions that can direct new experiments.

The current chapter gives the biological background, and a review of current computational approaches to model epithelial morphogenesis. In chapter 2, we analyze the effect of two-dimensional patterns of cell contractility on three-dimensional epithelial deformations using the 3D and the 2D model. We propose additional terms to the current 2D model framework to make it consistent with the 3D model. Finally, chapter 3 concludes this thesis, where we simulate dorsal appendage formation as an illustration of the modified 2D model framework and describe possible future directions to extend this work.

1.1 Epithelial morphogenesis

Epithelial tissue is one of the four types of tissue present in the adult organism; the others are connective, muscular and nervous tissue. In an adult, it forms the skin, inner linings of the organs, and walls of blood vessels. However, in a developing embryo, they play a much more dynamic role and act as the workhorses of structure formation [35]. In response to extracellular signals, epithelial sheet undergoes complex but regulated transformation to form three-dimensional structures.

In an epithelial monolayer, the cells divide their membrane into two different domains, apical and baso-lateral, that have distinct functional and structural components (Fig. 1.1A, [56]). Tight junctions and adherens junctions are present on the lateral surface, nearer to the apical side of sheet. Tight junctions keep the cells closely connected and the basal surface interacts with the basement membrane. Adherens junction interacts with the cytoskeletal component and forms an adhesive belt just below the apical surface [56]. Actin and myosin are part of the cell cytoskeleton and play an important role in cell shape changes. Actin is an ATP binding protein...
that can exist as a monomer or filaments and the myosin molecules can slide actin filaments over each other, thus producing local contraction of actin filaments [5].

Epithelial cells can change shapes using the molecular machinery available to them. A common cell shape change is induced by uniform constriction of apical surface that can drive localized bending. One of the well-studied examples of such a process includes blastula-to-gastrula transition across different species [33, 65]. Epithelial cells can also modulate the length of a single edge adjacent to two neighboring cells, sometimes leading up to cell intercalation. Multiple cell intercalation events lead to complex morphogenetic movements as observed during germ band ex-
tension during *Drosophila* embryogenesis [31] and notochord formation in ascidians [69]. *Drosophila* appendage formation is an interesting example where both bending and cell rearrangements work together to form three-dimensional structures [48].

## 1.2 Blastula-to-gastrula transition

A zygote, after fertilization, undergoes rapid cell division and forms a spherical ball of epithelial cells, known as blastula, which is filled with yolk and usually enclosed within a stiff membrane [33, 65]. Under these conditions, during early gastrulation, a patch of cells bends inward. This morphogenetic movement also helps in specification of distinct cell types from an initial group of similar cells [33, 65].

Epithelial cells that constitute the blastula are usually columnar in shape and the apical surface face the outside environment. In most cases, they undergo invagination driven by apical constriction of a patch of cells. For examples, *Drosophila* ventral furrow formation [54], *Nematostella* gastrulation [67], sea urchin primary invagination [29] and ascidian invagination [61]. Although, each species can have a very specific sequence of cell shape changes that leads to the formation of gastrula, the apical constriction was found necessary across all of them. Constriction of the apical surface leads to the expansion of the basal surface due to presence of the incompressible cytoplasm. Each cell now adopts a wedgelike shape that provides localized curvature to the epithelium and the blastula bends toward the basal side [39].

## 1.3 Computational approaches

An important part of understanding the physical and chemical mechanisms driving morphogenetic movements is the identification of forces that causes these changes. Developing a biophysical model for epithelium can help us identify the minimum external heterogeneity needed to drive such shape transformations. Thus differentiating
the genetic inputs from the passive mechanical response of the epithelium. Cell-based modeling approaches provide a framework to study epithelial morphogenesis that are driven by fine grained patterns of mechanical heterogeneity at the level of a single cell.

1.3.1 Cell-based models for blastula-to-gastrula transition

A variety of computational models, having different levels of complexities describing geometric and physical interactions of epithelial cells, have been proposed. One of the earliest studies to understand invagination through physical modeling were done by Lewis in 1947 [34]. He used brass bars to represent cell interface and rubber bands to simulate apical and basal forces and showed that differences in tension between two faces can drive bending of epithelia. Since then computational models have replaced
Figure 1.3: *Nematostella* blastula-to-gastrula transition. (A) *Nematostella* embryos at different stages of gastrulation. (B) Schematic highlighting cell geometry and model terms. Each cell is modeled as a polygon with 84 vertices. The cell cortex, intercellular connections, and apical contractile belt are modeled as linear springs. (C) Simulation results showing bottle cell formation, invagination, and zipper during gastrulation. Images are taken from [67].

Figure 1.4: *Drosophila* ventral furrow formation. (A) *Drosophila* embryos at different stages of furrow formation. (B) Cell-based model of the cross-section of the embryo at the onset of gastrulation. The cell in the ventral region are experiencing active apical constriction. (C,D) Simulation results showing lengthening of ventral cells and subsequent formation of closed and invaginated furrow. Images are taken from [52].
actual physical modeling. Odell et. al. [45] gave one of the first discrete cellular models representing early Drosophila embryo as a cross section of monolayer of cells, represented by viscoelastic elements. The model showed that apical constriction with an additional trigger mechanism for propagating the mechanical signal could lead to ventral furrow formation. This work inspired many subsequent computational studies to understand the mechanical origins of epithelial morphogenesis.

A recent study by Sherrard et al. [61] described ascidian endoderm invagination in great detail and proposed a cell-based model to explain the process (Fig. 1.2). A cell was modeled as a polygon, based on the cross-section of cells of the ascidian blastula. Each cell boundary was shared between two cells and was modeled as a collection of interconnected elements (Fig. 1.2B). The force terms in the model included tensile forces, spring forces on terminal nodes, constant cell volume term and repulsive term between edges to prevent edge intersection. Model showed that differential cortical tensions, consistent with the experimentally observed myosin patterns, could explain the dynamics of invagination (Fig. 1.2C).

A cell-based model, with similar features as discussed before, was proposed for the setting of blastula-to-gastrula transition in Nematostella (Fig. 1.3 [67]). Here, each cell was modeled as a complex polygon with 84 vertices and the edges were not shared between adjacent cells. Nodes from two cells were connected by springs. An additional term was introduced in the model to mimic filopodia connections, where adhesive forces between two random vertices, within a range, were activated for a limited interval of time (Fig. 1.3B). Model was able to successfully simulate the formation of bottle cells, invagination and zippering (Fig. 1.3C). Simulations showed that apical constriction and filopodia force was necessary to capture the dynamics of transition.

Another well-studied example of blastula-to-gastrula transition is the process of ventral furrow formation during early Drosophila embryogenesis (Fig. 1.4A). Two
recent studies used cell-based model, with different terms, to address the physical mechanism driving this process. Brezavšček et al. [6] constructed a 2D model to represent the cross-section of the blastula and defined an energy function that determined the equilibrium shapes. Each cell was modeled as a trapezoid with different line tension energy term for lateral, basal, and apical edges. Lateral edge was shared between two neighboring cells. Both, cell cytoplasm and yolk, was assumed incompressible and an outside membrane was modeled as an elastic shell. All cells with identical properties could produce infolding for region of parameter values. The simulation results are reminiscent of the buckling instability observed in an expanding surface, which is constrained laterally. The other recent study by Polyakov et al. [52] had a similar geometrical description of the blastula, but different energy terms to describe the epithelium. Here, instead of line tension terms for edges, each edge was modeled as a linear spring (Fig. 1.4B). The combination of apical constriction in a patch of cells along with reduction of basal rigidity was found sufficient to form a closed and invaginated furrow (Fig. 1.4C,D).

1.3.2 Vertex models

Vertex model is a term used to describe the off-lattice models in which each cell is represented as geometric objects [14]. In the 2D vertex model, epithelial cells
are approximated as a polygon, with vertices and edges shared between cells. A governing equation is defined for each vertex either by explicitly modeling the force on each vertex or by defining the energy of the sheet.

2D vertex models have been extensively used in past to understand epithelial morphogenesis in different biological contexts [14]. Classically, the 2D vertex model have been used to capture the aspects of epithelial morphogenesis that are constrained in two-dimensions (Fig. 1.5A, [14]). Some recent works include studies to understand the role of cell mechanics, cell neighbor exchange and cell division in determining network packing geometry in Drosophila wing disk [12]. The model captures the physical description of an epithelial monolayer, by taking into account the stretching energy, intercellular adhesive term, and contractility of actin-myosin ring present on the apical side. Cell neighbor exchanges can be easily included in the 2D vertex model framework [40].

Osterfield et al. [48] extended the 2D vertex model framework to simulate three-dimensional deformations. The model was developed to study dorsal appendage formation during Drosophila oogenesis. The vertices were allowed to move in three-dimensions and the gradient of energy and other geometrical factors were defined accordingly. Patterning of model parameters was based on the experimentally observed localization of myosin and was found sufficient to capture the dynamics of dorsal appendage formation. This work was extended by Murisic et al. [41], where it was shown that patterns of contractility, in the form of a ring, could drive out-of-plane deformations. They observed that sheet buckles symmetrically in both directions after a threshold in the line tension coefficient of edges belonging to the contractile ring.

Epithelial morphogenesis is essentially a three-dimensional process and so, 2D models are limited in its applicability and cannot capture many important biological processes. This motivates development of comprehensive 3D models for epithelium.
In the 3D vertex model framework, each cell is expressed as a polyhedron, with surface, vertex, and edge shared between neighboring cells. 3D vertex models have been used in past in the context of cell aggregates \[46, 47\], but have recently been adapted for an epithelium \[21, 39\]. Hannezo et al. \[21\] provided a theoretical framework that described an epithelial monolayer (Fig. 1.5B). The effective energy had contributions from cell-substrate interaction, cell-cell lateral interaction, actomyosin belt present on the apical side, and an incompressible cytoplasm. The model also had a term inhibiting the infinite spread of cells. The study explored the effect of model parameters, apical belt tension and surface tension parameters, on equilibrium cell shapes. For some parameter range, the aspect ratio of cells varied discontinuously and bistability in shapes was observed. An interesting consequence of this result is that epithelial cells can exist as squamous or columnar for similar intrinsic mechanical property. It is a minimal model and will need to be adapted in future to specific biological settings.
Chapter 2

Shape transformations of epithelial shells

This is work done in collaboration with Basile Audoly, Yannis Kevrekidis, and Stanislav Shvartsman and is published [39]. This chapter is a lightly edited version of the published manuscript.

2.1 Introduction

Regulated deformations of epithelial sheets play important roles during tissue morphogenesis and can be driven by a variety of mechanisms, the simplest of which rely on spatial patterns of apical cell contractility [31, 58, 74]. These prepatterns are commonly established by upstream signaling processes, which upregulate the activity of the actomyosin networks on the apical surfaces or apical edges in a subset of cells within the epithelium [37, 70]. Intracellular nonuniformities in contractility trigger shape changes of individual cells, leading to three-dimensional (3D) deformations, such as localized tissue invaginations. This sequence of processes, from patterning of apical contractility, to spatially restricted cell shape changes, to 3D tissue deformations has been documented in a wide range of experimental systems [61, 67, 48, 10, 52].
Figure 2.1: Schematic representation of the vertex model. (A) A model 3D cell with distinct apical, basal, and lateral surfaces. (B) Schematic highlighting different tension terms in the 3D vertex model. (C) Schematic explanation of the modified 2D vertex model. The modified 2D model idealizes the epithelial sheet as the midsurface (shown in black) of an epithelial monolayer with finite thickness $2h$ and assumes that a contractile segment (shown in red) joins the centers of the apical faces. (D) The figure shows two adjacent 2D cells $S_1$ and $S_2$ with shared edge $j$, cell centers ($\mathbf{C}_{S_1}$, $\mathbf{C}_{S_2}$) and unit outward normals ($\mathbf{N}_{S_1}$, $\mathbf{N}_{S_2}$), pointing towards the outer shell. $\mathbf{u}_j$ is the unit vector joining the cell centers and $h$ is the offset parameter. See section 2.3 for more details. The red line highlights a contractile segment that is offset by a distance from the surface along the cell normals.
A canonical example is provided by the early stages of mesoderm invagination in *Drosophila*, where apical constriction of cells on the ventral side of the embryo transforms a convex epithelial shell into a more complex shape with an omega-like cross-section [33]. In this system the apical surfaces of epithelial cells are facing towards the rigid membrane surrounding the embryo. In another well-studied experimental model, the developing *Drosophila* egg chamber, the epithelium has opposite polarity, with the apical surfaces oriented towards the oocyte, which is enclosed by the epithelial sheet [48, 3]. In this case, a two-dimensional (2D) patch of the follicle cells evaginates, bending in the direction of the membrane surrounding the egg chamber. This deformation is thought to be guided, at least in part, by the embedded contour of apically constricting epithelial cells.

The general principles governing 3D deformations induced by patterns of apical contractility remain poorly understood. It is in general unclear what determines the direction in which the patterned sheet is going to bend and whether the resulting deformation will be discontinuous or smooth. Several mathematical and computational models have been proposed to explore these questions, but none of them are sufficiently versatile for analyzing the interplay of multiple physical and geometrical factors [61, 67, 52, 45, 9, 7, 53, 26, 6]. For instance, multiple models have been used to describe the invaginations in the early embryos [61, 67, 52, 6]. However, most of these models consider only a 2D cross-section of the epithelial shell, which limits the class of shape transformations that can be analyzed.

Here we provide what appears to be a minimal, yet physically realistic computational framework that enables systematic exploration of epithelial deformations induced by spatial patterns of active and passive cell properties. Our approach is based on the recently proposed energy formulation in which every cell is modeled as a prism with apical, basal, and lateral surfaces that can have different properties [21]. We use this model to construct epithelial shells enclosing a fluid-filled volume
and surrounded by a hard membrane, mimicking a scenario encountered in developing tissues and tissue organoids. We then use numerical continuation algorithms to explore how these model epithelial shells deform in response to the prepatterns of apical contractility. We find that, for most cases, the deformations are smooth, i.e. they happen without bifurcations, when viewed as a function of the amplitude that characterizes the spatial pattern of apical contractility. We also demonstrate that a simpler 2D model, in which epithelial shells are constructed from polygonal cells, can describe the effects predicted by the 3D model and can be used to explore a wide range of morphogenetic processes.

2.2 3D vertex model

Vertex models constructed from 3D cells have been proposed to study epithelial morphogenesis in multiple experimental systems [21, 24, 47, 46, 4]. Based on these previous studies, we used the following energy functional to model an epithelial monolayer constructed from cells with distinct apical and basal surfaces (Fig. 2.1(A,B)):

$$E_{3D} = \sigma \sum_e l_e + \alpha \sum_l S_l + \gamma \sum_b S_b + B \sum_c (V_c - V^0_c)^2. \quad (2.1)$$

In this expression, the first term sums over all apical edges $e$, the second term sums over lateral surfaces $l$, the third term sums over basal surfaces $b$, and the last term sums over cells $c$. The first term corresponds to the line tension along the apical edges of neighboring cells; $l_e$ is the edge length and $\sigma$ is the apical line tension coefficient. The second and third terms correspond to the contributions from lateral and basal tensions, which are proportional to the lateral and basal surface areas ($S_l, S_b$) with coefficients $\alpha$ and $\gamma$, respectively. These coefficients model the resistance to deformation due to the cytoskeletal meshwork that underlies the surface. The last term penalizes the deviation of the cell volume, $V_c$, from its target value, $V^0_c$. 

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with compression modulus $B$ to approximate cytoplasmic incompressibility. For more details see appendix A.1.A.2.

In our 3D model, we define prepatterned apical edges to represent a contractile ring $R$ embedded in the apical surface (shown in red in Fig. 2.2(A1), 2.3(A1)) or an apically constricting patch of cells $P$ (shown in red in Fig. 2.2(B1), 2.3(B1)). The first pattern is motivated by the embedded ‘compressive cable’ observed in follicle cells during Drosophila appendage formation and early sea urchin embryo, which displays localization of the motor protein myosin [48, 43]. The second pattern is motivated by biological settings in which a group of cells are influenced by uniform actomyosin constriction across the apical surface [58, 38, 36]. Apical edges belonging to the contour or the patch are assigned an apical line tension ($\sigma + \Gamma$), which is larger than the apical line tension $\sigma$ of the other non-patterned edges. This is implemented through an additional energy term for the prepatterned edges

$$E_\Gamma = \Gamma \sum_{i \in R \text{ or } P} l_i, \quad (2.2)$$

where the index $i$ runs over all edges belonging to the apical ring $R$ or apical patch $P$.

### 2.3 2D vertex model

Most of the previously published 2D vertex models were used to describe the dynamics of epithelia constrained to two spatial dimensions [12, 12, 14]. More recently, we have used these models to explore 3D deformations induced by prepatterning of cell properties ([48, 10, 41], Fig. 2.4). We extended these models so that they can account for the natural curvature of the sheet, and, importantly, for the intrinsic apicobasal polarity of epithelial cells. The energy in this model is defined in the following way:
\[ E_{2D} = \mu \sum_c (A_c - A_c^0)^2 + \sigma \sum_j l_j + \beta \sum_{j'} \left( (1 - \mathbf{N}_{s2(j')} \cdot \mathbf{N}_{s1(j')}) + k_{j'} (\mathbf{N}_{s2(j')} - \mathbf{N}_{s1(j')} \cdot \mathbf{u}_{j'}) \right). \]  

The first term sums over all cells \( c \), the second term sums over all edges \( j \), and the last term sums over all interior edges \( j' \), i.e. edges shared by two adjacent cells \( s1(j') \) and \( s2(j') \). The first term corresponds to sheet elasticity, where \( A_c \) is the area of cell \( c \), \( A_c^0 \) is its target area, and \( \mu \) is the stretching modulus. The second term captures intercellular interactions in the form of tensile forces along cell-cell edges, where \( l_j \) is the length of the edge \( j \) and \( \sigma \) is the line tension coefficient. The last term represents the bending energy term; where \( \beta \) is the bending elasticity coefficient and \( k_{j'} \) represents the local natural curvature of the shell (\( k_{j'} \) is roughly the rest value of the dihedral angle between the faces adjacent to edge \( j' \) and it scales like the natural curvature of the midsurface times the typical cell size; see appendix B.1 for details). Here \( \mathbf{N}_s \) denotes the ‘outward’ unit normal to a cell \( s \) (i.e. the normal vector of each cell points towards the ‘outside’ of the closed shell). \( \mathbf{u}_{j'} \) is the unit vector joining the cell centers (Fig. 2.1(C,D)),

\[ \mathbf{u}_{j'} = \frac{\mathbf{C}_{s2(j')} - \mathbf{C}_{s1(j')}}{|\mathbf{C}_{s2(j')} - \mathbf{C}_{s1(j')}|}. \]  

where \( \mathbf{C}_{s2(j')} \) and \( \mathbf{C}_{s1(j')} \) are cell centers, defined as the mean position of the nodes belonging to the cells \( s1 \) and \( s2 \), respectively (Fig. 2.1(C,D)). The bending energy term defines an ‘at rest’ value of the angle between the normals of adjacent cells, which depends on \( k_{j'} \) (and is proportional to \( k_{j'} \) for small \( k_{j'} \) and hence implements the notion of natural curvature. For more details see appendix B.1,B.2.
In our 2D model, we idealize the epithelial sheet as the midsurface of a 3D epithelial monolayer (Fig. 2.1(C)). We proceed to show how we mimic the effect of the prepatterns in our 2D model that are present on the apical surface of an epithelium (Fig. 2.1(D)). From now on, we will refer to this representation of prepatterns as being offset from the midsurface. Such prepatterns include a contractile ring $R$ (Fig. 2.5(A1)) and a constricting patch $P$ (Fig. 2.5(B1)): a contractile ring $R$ is defined by a closed path joining the centers of a ring of adjacent cells (Fig. 2.5(A1)), while a constricting patch $P$ represents a subset of the mesh and is implemented by adding a line tension along all segments joining the adjacent cell centers that are contained within this subset (Fig. 2.5(B1)). Segments belonging to either type of prepattern are assigned a line tension $\Gamma$ and an offset parameter $h$, and an additional energy term is considered:

$$E_\Gamma = \Gamma \sum_{i \in R \text{ or } P} \left( |C_{s2(i)} - C_{s1(i)}| - h(N_{s2(i)} - N_{s1(i)}) \cdot u_i \right). \quad (2.5)$$

where the index $i$ runs over all edges belonging to the apical ring $R$ or apical patch $P$, and $h$ is the half the thickness of the real tissue.

The key feature of equation 2.5 is that it represents the line tension of segments that are offset by a distance $h$ from the midsurface (see appendix B.2 for details), but does so by using solely the degrees of freedom of the 2D model, namely the positions of the nodes lying onto the midsurface. The line tension parameter $\Gamma$ acts in two ways: it brings the cell centers closer together and, when $h \neq 0$, it bends the surface so that the end points of the normal vectors come close together ($h > 0$) or further apart ($h < 0$). Note that the line tension prepatterning parameter $\Gamma$ in the model with 3D cells has qualitatively similar effects.

In the special case $k_{j'} = 0$ and $h = 0$, this model reduces to the 2D vertex model which has been used in the literature to understand epithelial deformations for flat epithelial sheets in different contexts [48, 11, 12, 41]. Hereafter, we will refer to that
particular case as the naturally planar 2D vertex model (since $k_{j'} = 0$, the bending energy term corresponds to a naturally flat sheet). Note that the new terms which we introduce in the present work, namely the one proportional to $k_{j'}$ in Eq. 2.3 and the one proportional to $h$ in Eq. 2.5 both depend on the nodal positions through the same expression $(N_{s2(j')} - N_{s1(j')}) \cdot u_{j'}$. As a result, both terms have similar numerical implementation.

2.4 Modeling the effects of constraints

To study the effects of epithelial shell curvature, a fluid-filled inner cavity and an outer stiff membrane during epithelial bending, we consider an initial homogeneous spherical configuration and include additional terms in the energy functions:

$$E = B_Y (V_Y - V_Y^0)^2 + \epsilon \sum_k \frac{1}{(R_C - R_k)^n} + E_{2D/3D} + E_\Gamma \tag{2.6}$$

Here, the first term penalizes deviations from the initial volume of the inner cavity. $V_Y$ is the volume enclosed by the closed surface, $V_Y^0$ is the initial volume, and $B_Y$ is a compression modulus. The second term corresponds to the outer membrane stiffness that runs over all vertices on the outer surface of the shell and restricts the radial motion of vertices within a sphere of radius $R_C$. This sphere is concentric with the homogeneous spherical configuration from which the system is initialized. The center of the initial spherical configuration acts as reference center to calculate the radial distance of the vertices. The term $R_k$ denotes the radial distance of the vertex $k$, and hence $R_k - R_C$ represents the membrane thickness near that vertex. $\epsilon$ is a membrane stiffness parameter, and $n$ is the exponent of the repulsive potential term that models the effects of the outer stiff membrane.
2.5 Numerical methods

To find an equilibrium shape for a typical configuration in the 3D vertex model, we solved the system of nonlinear algebraic equations that correspond to vanishing of the gradient of the energy with respect to node positions by Newton-Raphson iteration. The number of equations was halved for the corresponding configurations in the 2D vertex model. Initial guesses were obtained by direct forward Euler integration following overdamped gradient dynamics. In some cases, rotational and translational symmetries had to be factored out through appropriate pinning conditions, as described in appendix C. Pseudoarclength continuation [27, 44] was used to follow solution branches in parameter space and to go around turning points. The eigenvalues of the Jacobian upon convergence quantify the stability of the computed equilibrium shapes. Branches were terminated when equilibrium solutions featured vertices too close to each other (edge length < 0.01). The merging of vertices and the rearrangement of cell neighbors were not considered here. We used the Armadillo, a C++ linear algebra library, to solve the linear systems of equations and calculate leading eigenvalues [57].

2.6 Homogeneous configurations and model parameters

In all cases considered below, we start with a system with spatially uniform cell properties at a mechanical equilibrium. This equilibrium configuration is then used as a starting point for numerical continuation that analyzes the effects of spatial patterns of cell properties, with the amplitude of the pattern chosen as continuation parameter. To construct the initial equilibrium states for our model epithelial shells, we analyzed an idealized system sheet with uniform cell properties. For the 2D
vertex model, we considered a shell constructed from regular hexagons (Fig. 2.1(D)). For the 3D model, we considered a shell constructed from a ‘lampshade’ shape with hexagonal apical/basal surfaces (Fig. 2.1(A)). In both these cases, one can analyze the explicit expressions for the overall energy as a function of the geometric and model parameters. The geometric parameter for the 2D model is the edge length of the unit hexagonal cell. For the 3D model, the geometric parameters are the edge length of the hexagonal apical and basal surfaces and the height of the unit cell. The minima of such functions provide a relationship between equilibrium cell shape and tissue properties.

Once the parameters were found for an idealized sheet at an equilibrium, we used DistMesh to realize an epithelial shell with a finite number of cells tiling a closed surface \[50\]. This meshing package builds triangular tessellations on a sphere for the given edge length. As an input geometry for the 2D vertex model, we used these tessellations to construct polygonal cells by considering centers of triangular mesh elements as vertices of epithelial cells. To construct the 3D vertex model input geometry, we first constructed the inner surface of the epithelial shell using the 2D model input geometry approach and then extended the vertices radially to a given height to form 3D cells. This produces a globally curved epithelial shell with finite number of 3D ‘lampshade-shaped’ cells, i.e. cells with asymmetric apical and basal surface. Further details are provided in the appendix A.3.

2.7 Results

2.7.1 3D vertex model

3D vertex models have previously been used to study dynamic tissue morphogenesis for cell aggregates and spatially uniform cell sheets \[47, 46, 42\]. Recently Hannezo et al. \[21\] extended a similar framework to model different shapes of epithelial cells.
Figure 2.2: 3D deformations induced by prepatterns of line tension in a model with 3D cells for the *apical-in* case. (A1-B1) Initial configurations, showing the inner surface (i.e. apical side and omitting the outer basal surface) of the shell, with different heterogeneities: (A1) Contractile ring. (B1) Patch of apically constricting cells. (A2-B2) Representative evaginated states, showing the inner surface, where the enclosed patch bends outward (positive deflection, $\delta > 0$, as defined in Fig. D.1). (A3-B3) Cross-section representation of equilibrium shapes (A2-B2) respectively. (A4-B4) Steady state diagrams showing deflection $\delta$ with increasing parameter $\Gamma$. Solid line: stable steady states. Cross-sections of representative steady states (A1-B1, A2-B2) are shown as insets. Parameter values are listed in Table E.1.
Here, we use a simplified 3D vertex model (Eq. 2.1), along with the additional term for prepatterns (Eq. 2.2), to numerically find equilibrium shapes in an epithelial monolayer induced by the in-plane prepatterns of apical contractility. We also analyze the effects of a fluid-filled cavity and an outer membrane (Eq. 2.6) on out-of-plane deformations induced by such prepatterns. All of these factors are present in different developmental contexts including *Drosophila* appendage formation [3, 49] and the blastula-to-gastrula transition in different species [61, 67, 52, 6, 65, 54].

To understand the effect of model parameters on cell morphology and to provide good initial guesses for the numerical calculation of equilibrium shapes, we calculated equilibrium shapes for flat, as well as curved homogeneous epithelial monolayers. The analysis was performed for ideal cases where apical and basal surfaces were approximated as hexagons. For the 3D vertex model with planar cells, increasing the lateral tension coefficient ($\alpha$) makes the equilibrium cell shape more columnar, whereas increasing the basal tension coefficient ($\gamma$) makes the cell more squamous (Fig A.2(C, D)). We discuss the effects of basal and lateral tension coefficients in greater detail in appendix A.3. We use this information to construct the initial homogeneous configuration ($\Gamma = 0$) with given model parameters and then apply different prepatterns ($\Gamma > 0$) and solve for the equilibrium shapes.

We started with a closed shell monolayer of 3D cells, which had polarity similar to the cells in the follicular epithelium in the developing *Drosophila* egg, where the apical side forms the inner surface of the epithelium (apical-in case, Fig. A.1(D)). Consistent with recent observations [18], we kept the value of the cell-volume compression modulus $B$ large, to make sure that the deviation from the initial cytoplasmic volume of each cell was small. We then introduced spatial patterns of apical contractility. We considered two prepatterns of apical constriction in different settings: (I) an embedded contractile ring (Fig. 2.2(A1)); and (II) a patch of apically constricting cells (Fig. 2.2(B1)). As mentioned before, the first prepattern is motivated by the
study of *Drosophila* appendage formation [48]. The second prepattern is found in diverse developmental settings in which a group of cells undergo apical constriction driving localized tissue bending [58, 38, 36].

We found one continuous branch of stable equilibria in which the epithelium bent in the basal direction (evagination) for both apical contractility prepatterns (Fig. 2.2(A4,B4)). Due to cytoplasmic incompressibility, a patch of cells undergoing apical constriction leads to the expansion of the basal surface and thus causes the sheet to bend in the apical-to-basal direction. Inclusion of an outer membrane term kept the configuration more spherical (Fig. A.4), and increasing the inner-fluid compression modulus ($B_Y$) decreased deflection without affecting the qualitative nature of the equilibrium shapes (Fig. A.5).

We then considered a scenario in which the apical side forms the outer surface of the shell (*apical-out* case, Fig. A.1(C)) by reversing the polarity and keeping the energy formulation the same. A similar scenario is observed during the blastula-to-gastrula transition in many species, such as sea urchin [43] and *Drosophila* [54]. We introduced similar prepatterns of apical contractility as before (Fig. 2.3(A1,B1)) and found one continuous branch of stable steady states for the case of a patch of apically constricting cells in which the epithelial shell formed an invagination (Fig. 2.3(B4)). However, in the case of prepatterning with a contractile ring, we observed bistability in the shapes of shells (Fig. 2.3(A4)). This shape bistability was not present in configurations with smaller shell curvature (Fig. A.7) and with a one-cell wide ring (Fig. A.6). We also considered a prepattern of line tension in the form of a two-cell wide ring of apically constricting cells, which was inspired by the pattern of actomyosin localization during primary invagination in the sea urchin embryo [43, 29]. Here, as well, we found one continuous branch of stable invaginated shapes (Fig. 2.3(C4)).
To summarize, in response to most of the analyzed patterns of apical contractility, the 3D model predicts a smooth transition to the evaginated state when the apical surface forms the inner surface and to the invaginated states when the apical surface forms the outer surface of the shell.

2.7.2 Naturally planar 2D vertex model

Naturally planar 2D vertex models provide a computationally simpler framework to model epithelial deformations, while still incorporating the essential physical features of the apical surface of the epithelium that drive such transformations. In this section we determine what aspects of the 3D model can be captured with a naturally planar 2D vertex model. We use the model presented by Murisic et al. [41] as our starting point. This model can be viewed as a special case of the 2D model introduced here \((h = 0; k_j' = 0)\).

Similar to our analysis of the model with 3D cells, we examined the case of a closed shell with different prepatterns of apical cell contractility. In contrast to the 3D model, the naturally planar model showed no out-of-plane deformations for a constricting patch prepattern. However, for a contractile ring, we found two disconnected branches of steady state solutions (Fig. 2.4). Equilibrium shapes with cells deflecting outward (evaginated state) formed a continuous branch of steady states from an initially homogeneous configuration, whereas solutions with cells deflecting inward (invaginated state) formed a disconnected branch (Fig. 2.4(E)).

This steady state diagram can be traced back to the pitchfork bifurcation observed by Murisic et al. [41] in the presence of contour forces for flat configurations. However, the pitchfork bifurcation here is imperfect, as the sheet curvature breaks the symmetry present in the flat configuration case (Fig. B.2). We observed that the turning point of the branch of invaginated states shifts to the right as the curvature of the initial homogeneous configuration is increased. This is consistent with the past analysis.
Figure 2.3: 3D deformations induced by prepatterns of line tension in a model with 3D cells for the *apical-out* case. (A1-C1) Initial configurations, showing the outer surface (i.e. apical side) of the shell, with different heterogeneities: (A1) Contractile ring. (B1) Patch of apically constricting cells. (C1) A ring of apically constricting cells, two cells wide. (A2-C2) Representative invaginated states, showing the outer surface, where the enclosed patch bends inward (negative deflection, $\delta < 0$). (A3-C3) Cross-section representation of equilibrium shapes (A2-C2) respectively. (A4-C4) Steady state diagrams showing deflection $\delta$ with increasing parameter $\Gamma$. Solid (dashed) line: stable (unstable) steady states. Cross-sections of representative steady states (A1-C1, A2-C2) are shown as insets. Parameter values are listed in Table E.1.
Figure 2.4: 3D bending of closed epithelial shells in the naturally planar 2D vertex model due to the embedded contractile ring. (A) Initial equilibrium configuration with the contractile ring (shown in red). (B) Representative invaginated state, where the enclosed patch bends inward (negative deflection, $\delta > 0$). (C) Representative evaginated state, where the enclosed patch bends outward (positive deflection, $\delta > 0$). Dashed red line represents patterned edges that lie behind the evaginated patch. (D) Representative unstable equilibrium configuration. (E) Steady state diagram showing deflection $\delta$ with increasing parameter $\Gamma$. Solid (dashed) line: stable (unstable) steady states. Insets (I) and (II) highlights the breakup of the pitchfork bifurcation in the naturally planar 2D vertex model due to curvature (discussed more in section B.3). Cross-sections of representative steady states (A-D) are shown as insets. Parameter values are listed in Table E.2.
of buckling in Elastica (a thin strip of elastic material) with imperfections, such as preferred curvature or misaligned axial load [68, 2, 72].

The presence of an outer membrane term tends to keep the configuration more spherical (Fig. B.3 A,B). As expected, increasing the inner-fluid compression modulus ($B_Y$) and bending elasticity coefficient ($\beta$) made it more difficult to bend the epithelium. It decreased the deflection of evaginated shapes for a given value of $\Gamma$ and shifted the limit point to the right (Fig. B.3 D,E). We observed similar imperfect pitchfork bifurcations for different stretching moduli $\mu$.

In sum, we observed a typical imperfect pitchfork bifurcation for the closed shell configuration. The addition of an inner fluid-filled cavity and outer membrane term does not affect the qualitative response of the naturally planar 2D vertex model, influencing only the domain of shape bistability. The results of the naturally planar 2D vertex model suggest that the initially curved and homogeneous epithelium can smoothly bend, due to contour forces, only in the outward direction, which is similar to the response of the apical-in case of the 3D model to a contractile ring. Thus, this model does not capture all of the effects predicted by the 3D model, particularly the deformations driven by an apically constricting patch.

### 2.7.3 2D vertex model

As noted above, the naturally planar 2D vertex model cannot capture different aspects of the 3D model, including apicobasal polarity, natural curvature of the sheet, and a smooth transition from homogeneous configuration to invaginated states in the presence of an apical constriction pattern. Here we show that the 2D model with natural curvature, as introduced in eqs. 2.3-2.5, is fully consistent with the 3D model responses to different prepatterns, while maintaining the simpler framework of the 2D model. The model includes additional terms making the sheet naturally curved and
Figure 2.5: 3D deformations induced by prepatterms of line tension in a model with 2D cells for the apical-in case. (A1-B1) Initial configurations with different heterogeneities: (A1) Contractile ring, (B1) Constricting patch. Both are implemented as subsets of the dual mesh, which is used to offset contractile patterns; see text for details. (A2-B2) Representative evaginated states. (A3-B3) Steady state diagrams showing deflection $\delta$ with increasing parameter $\Gamma$ corresponding to the prepatterning defined in (A1-B1) respectively. Solid line: stable steady states. Cross-sections of representative steady states (A1-B1, A2-B2) are shown as insets. Parameter values are listed in Table E.3.
incorporates apicobasal polarity by offsetting the prepatterns away from the sheet’s midsurface such that they lie on the apical side of the shell.

Similar to previous sections, we considered two prepatterns of apical contractility: a contractile ring (Fig. 2.5(A1)) and a constricting patch (Fig. 2.5(B1)), now offset from the 2D sheet. We started with a homogeneous closed shell configuration at equilibrium, for which we set the value of $k_j'$ equal to the average angle between the normals of the adjacent cells (see appendix B.1 for details). We then introduced the prepatterns of constriction in two settings corresponding to apical-in ($h < 0$) and apical-out ($h > 0$) cases (note that we defined the cell normals in both cases such that they always point ‘outwards’ with respect to the shell). For the apical-in case, we found that each prepattern produces a continuous branch of equilibrium shapes as we increased $\Gamma$ in which the epithelium bent in the ‘outward’ direction (evaginated state) (Fig. 2.5(A3,B3)). For the apical-out case, the constricting patch prepattern produces one continuous branch of equilibrium shapes with the epithelium bending in the ‘inward’ direction (invaginated state) (Fig. 2.6(B3)). However, we observed bistability in the shapes of shells in the case of the contractile ring prepattern (Fig. 2.6(A3)). Increasing the inner-fluid compression modulus ($B_Y$) decreased deflection without affecting the qualitative nature of steady state diagram (Fig. B.5). Thus, the results obtained with the 2D vertex model are qualitatively similar to results from the 3D vertex model, suggesting that the additional terms can effectively capture the features of the complete 3D description of the epithelial sheet and its responses to different patterns of apical cell contractility.

2.8 Discussion

We used vertex models to explore how the spatial patterns of cell contractility lead to 3D deformations of epithelial shells. Previous studies of such deformations used
appropriately modified elastic shell theories to make connections between biophysical descriptions of epithelia and a large body of tools and ideas from continuum mechanics \[7, 26, 23, 30\]. Although these studies can mimic a number of canonical processes, including vertebrate neurulation, they are difficult to adapt to different biological systems, such as morphogenesis driven by cell rearrangements, directed migration, or cell proliferation \[65\]. Furthermore, the spatial patterns of apical contractility that drive 3D epithelial deformations are commonly fine grained. For instance, the early stages of sea urchin gastrulation are triggered by a contractile ring that is only two
cells wide [43]. These observations provide a clear motivation for models that can resolve individual cells.

A number of cell-based models, similar to the vertex models used in our work, have been used to describe deformations in 2D cross-sections of epithelial tissues [61, 67, 14, 41]. The most extensive work has been done to model the formation of the ventral furrow in the early *Drosophila* embryo [52, 54]. We extended these models to three dimensions, making it possible to investigate deformations induced by a broader class of spatial patterns of apical contractility. Our results can be summarized as follows. Model epithelia constructed from 3D cells, with distinct apical and basal properties, can readily generate both invaginated and evaginated shapes. The direction of bending is dictated by the apicobasal orientation of the epithelium: cells with increased apical contractility bend out of the sheet in the apical-to-basal direction. In contrast, naturally planar 2D vertex models predict that two different equilibrium shapes (invaginated and evaginated) coexist for the same set of parameters. The invaginated state exists as an isolated branch, which cannot be reached by continuous changes in the parameters of the spatial prepatterns; this is in contrast to the predictions of the 3D model. It also lacks the ability to include the apicobasal polarity or the natural curvature of the epithelial monolayer.

To address these issues, we proposed a modified 2D vertex model that better accounts for the 3D nature of the epithelium. This was accomplished by incorporating additional terms in the energy function. These terms effectively offset contractile patterns from the sheet and include the natural curvature of the epithelium. The modified model can readily capture the full effects of the 3D model. Most importantly, it can generate both evaginated and invaginated shapes depending on the apicobasal polarity of the epithelial shell. This suggests that the modified 2D vertex model can be used to describe shape transformations in a broader class of systems, especially those in which 3D deformations are driven by asymmetric apical contractility patterns.
while maintaining the computational simplicity of the 2D framework. In our current work, we are using these models to explore shape transformations that include cell rearrangements and directed cell migration.
Chapter 3

Complex structures from patterned cell sheets

The following sections are a lightly edited version of the submitted manuscript, which includes work done in collaboration with Basile Audoly, and Stanislav Shvartsman [40].

3.1 Introduction

It is believed that the organization of the first multicellular animals resembled an envelope, a structure formed by two epithelial layers, with the lower layer adhering to the external surface and top layer exposed to the external environment [1]. Such two-layered organization is observed in *Trichoplax adhaerens*, one of the simplest extant metazoans, an organism which can be studied in the laboratory [59]. This organism moves along the surface in amoeboid fashion in search of food and can bend in the direction normal to the surface to engulf particles [64]. A similar class of shape transformations, involving in-plane reshaping and out-of-plane bending, is observed in the early embryos of all animals, from ascidians to humans. In particular, at an early stage of development the embryo forms a blastula, which comprises
Figure 3.1: 2D vertex model and patterning. (A) Schematic highlighting mechanical contributions of area elasticity and line tension terms in the vertex model (Eq. 3.1).
(B) Dependence of the cell side length $l_c$ on the area elasticity coefficient $\mu_s$ for a uniform tissue at mechanical equilibrium. Solid (dashed) line: stable (unstable) equilibrium solutions. A representative flat homogeneous configuration is shown as an inset. (C) A representative configuration showing in-plane deformations due to spatial patterning of model parameters. (D) A representative configuration showing out-of-plane deformations due to patterning of model parameters. (E,F) Schematic of cell shape changes during bending. Each cell adopts a wedge-like shape due to apical constriction, leading to the bending of an initially flat sheet. (G) Schematic of a T1 transition event, in which a vanishing edge is resolved into a new edge reflecting new local cell connectivity (described in greater detail in Appendix 3.6).
an epithelial sheet enclosing a fluid-filled cavity. As embryogenesis proceeds, more complex structures are formed by progressive bending and in-plane rearrangements of epithelial subdomains [65, 35]. Imaging studies across species have revealed that these events are driven by controlled shape changes of individual epithelial cells. By controlling the activity of actomyosin networks, these cells can control their apical areas and lengths of individual edges [60, 36].

The molecular machinery regulating these processes is incredibly complex, but the general principles of morphogenesis driven by spatiotemporal control of actomyosin and cell adhesion systems have become progressively understood, to the point that we can now recognize the same functional subroutines in diverse experimental models. For instance, localized apical constriction can induce localized bending of epithelial sheets [36]. Among the numerous examples of this well-studied process are mesoderm invagination in Drosophila [33], primary invagination in Ciona intestinalis [61], and early stages optic cup formation in Xenopus [17]. In all of these cases, cells within the invaginating region constrict their apical surfaces (Fig. 1.1C). A finer mode of control is needed to induce in-plane reshaping of epithelial sheets during the convergent extension movements observed in animal gastrulation [66]. As was shown by studies of the germband extension in Drosophila [55] and Xenopus gastrulation [63], this type of morphogenesis relies on the regulation of only a subset of cell edges within the epithelium. In these cases, increased contractility of a subset of cell edges across the sheet triggers cell intercalations, leading to in-plane reshaping of an epithelial subdomain (Fig. 1.1D).

A combination of in-plane reshaping of epithelial subdomains and their out-of-plane bending can generate a wide range of three-dimensional multicellular shapes. Computational models of abstracted mechanisms can be used to both explore the functional capabilities of a class of mechanisms and test the feasibility of mechanisms proposed to explain the dynamics observed in specific developmental systems. Start-
ing from the seminal papers by Odell and co-workers [45], cell-based computational models have been used successfully for both of these purposes. However, most existing models focus either on out-of-plane bending or on in-plane cell shape changes and rearrangements. The first class of models used to mimic epithelial invaginations and evaginations relies on cells with distinct apical and basal surfaces [61, 6, 52, 67]. Most of these models are essentially two-dimensional (describing the cross-section of a deforming epithelial shell), designed to capture the emergence of the characteristic Ω-like shapes during early gastrulation. In the second class of models, designed to explore in-plane deformations, cells are approximated by convex polygons that can deform and exchange neighbours but are always confined to the flat surface [42, 12, 13].

In our recent work we have developed a model that can be used to describe both in-plane cell constriction and out-of-plane bending of epithelial sheets [39]. This model can be used to systematically explore the connections between two-dimensional patterns of cell contractility and the resulting three-dimensional tissue deformations. Here we show how the same model can be extended to include spatially controlled cell rearrangements. In the next section, we review the key aspects of the mathematical formulation and numerical implementation of the model. After this, we demonstrate how the model be used to explore the multicellular dynamics involved in the three-dimensional morphogenesis of the respiratory appendages on the Drosophila eggshell, an experimental system in which morphogenesis can be studied using genetic, imaging, and modeling approaches [3, 48]. We conclude by discussing the limitations of the proposed model and outline directions for future work.

3.2 Physical description of an epithelial sheet

The starting point for our computational analysis of 3D epithelial morphogenesis is a model in which the epithelium is modelled as a flat sheet constructed from
polygonal cells \[42, 12, 14, 10\]. The energy of the system depends on the geometrical configuration of the vertices of the polygons through the areas of cells and lengths of cell-cell edges:

\[ E_{2D} = \sum_s \mu_s (A_s - A_0^s)^2 + \sum_j \mu_j l_j. \] (3.1)

The first sum in equation 3.1 runs over all cells \(s\) and penalizes the deviation of each cell’s area \(A_s\) from a target value \(A_0^s\), with stretching modulus \(\mu_s\). The second sum runs over all edges \(j\), representing an effective line tension, where \(l_j\) is the length of each edge and \(\sigma_j\) is a line tension coefficient. This term models the joint effect of cell-cell adhesion and actomyosin contractility (Fig. 3.1A).

In a uniform tissue at mechanical equilibrium, each cell in the model epithelium is a hexagon whose side length is determined by the balance of the area and edge energies (Fig. 3.1B). However, when the tissue is patterned, cell shapes can change as a function of position (Fig. 3.1C). When the vertices are allowed to move in three dimensions, some of the two-dimensional patterns of cell properties can cause out-of-plane deformations (Fig. 3.1D). For instance, contractile contours, which are implemented by increasing the line tension along the edges forming a ring around the patch of cells, can lead to a pitchfork bifurcation, in which a flat configuration loses stability with respect to out-of-plane displacements \[41\]. Specifically, when all cells within the patch increase their edge tension, cell shapes within the patch and its surroundings change, but the sheet remains flat. This is in contrast to what is observed in a wide range of experimental and computational systems, in which apical constriction of a patch of cells causes localized tissue bending \[36\].

The bending of an epithelium due to apical constriction is commonly described by models in which cells have distinct apical and basal surfaces and the volume of each cell is conserved \[61, 45, 6, 52, 67, 21, 47\]. In these models, constriction of the apical surface leads to expansion of the basal surface due to the incompressibility of
the cell. Each cell thus adopts a wedge-like shape that provides localized curvature to the epithelium, which bends towards the basal side (Fig. 3.1E,F). In our recent work [39], we found that these effects can be captured by adding a new term to the energy function in the model with polygonal cells. Specifically, the cell sheet is modelled as the midsurface of an epithelial monolayer (Fig. 2.1C). The additional term mimics the effect of the contractile segments that are offset from the midsurface, reflecting cell contractility on the apical surface of the epithelium.

The vertices of these segments are at distance $h$ from the cell centers along the cell normals. The energy for such offset segments can be defined as

$$\widetilde{E}_\Gamma = \Gamma \sum_i \left| (C_{s2(i)} + hN_{s2(i)}) - (C_{s1(i)} + hN_{s1(i)}) \right|, \quad (3.2)$$

where the index $i$ represents the edge shared by two adjacent cells $s1(i)$ and $s2(i)$, $C_s$ denotes a cell center, defined as the mean position of the vertices belonging to cell $s$, $N_s$ denotes the unit normal to that cell $s$, $\Gamma$ characterizes the line tension along the segments joining the cell centers, and $h$ represents the half-thickness of the epithelial monolayer. In short, $\widetilde{E}_\Gamma$ is the line tension energy term for the segments joining the points $(C_s(i) + hN_{s(i)})$. We only keep the linear truncated form of $\widetilde{E}_\Gamma$ (eq. 3.2) and add it to the energy defined in eq. 3.1 to define the total energy of the epithelial sheet. The truncated form is given by

$$E_\Gamma = \Gamma \sum_i \left( |C_{s2(i)} - C_{s1(i)}| - h(N_{s2(i)} - N_{s2(i)}) \cdot u_i \right), \quad (3.3)$$

where $u_i$ is the unit vector joining the cell centers:

$$u_i = \frac{C_{s2(i)} - C_{s1(i)}}{|C_{s2(i)} - C_{s1(i)}|}. \quad (3.4)$$

The total energy of the system is given by $E = E_{2D} + E_\Gamma$ (eqs. 3.1,3.3).
As $\Gamma$ is increased, the sheet favors the configuration where the cell centers are closer together. Additionally, when $h \neq 0$, an increase in $\Gamma$ brings the endpoints of the normal vectors close together ($h > 0$) or further apart ($h < 0$), causing the sheet to bend. This can be appreciated from analyzing the following setting, which assumes that $\mathbf{N}_{s1}$, $\mathbf{N}_{s2}$ and $\mathbf{u}_i$ are coplanar for the sake of simplicity (Fig. 2.1D). Consider two cells $s_1$ and $s_2$ adjacent to a particular edge $i$. Let $\theta$ be the angle between the normals $\mathbf{N}_{s1}$ and $\mathbf{N}_{s2}$ and assume that the unit vector $\mathbf{u}_i$ makes an angle $(\pi - \theta)/2$ and $(\pi + \theta)/2$ with $\mathbf{N}_{s1}$ and $\mathbf{N}_{s2}$, respectively. In the additional term (Eq. 3.3), the contribution from this segment for the case $\theta \ll 1$ is proportional to $\Gamma(l_s + h\theta)$, where $l_s$ is the distance between the cell centers, $\mathbf{C}_{s1}$ and $\mathbf{C}_{s2}$, adjacent to the edge $i$. From the simplified expression, it is more apparent that to minimize energy in the presence of the additional term, the sheet can now constrict (decrease $l_s$) as well as bend (increase or decrease $\theta$, depending on the sign of $h$).

The modified 2D model successfully captures the essential behaviour of the 3D model [39]. For example, the epithelium bends only in the apical-to-basal direction in response to the two apical contractility patterns that we considered: a contractile ring or a uniform constricting patch of cells. The epithelial sheet smoothly deforms into either invaginated or evaginated state in the presence of the apical contractility patterns, depending on the direction of apicobasal polarity. The same effects are preserved when the additional features, including natural curvature of the sheet, enclosed fluid, are added to the model. Finally, the same model can be used to describe dynamics of cell and tissue deformations. This is done using an overdamped setting, in which the forces acting on each vertex are calculated by taking the partial derivatives of the energy function with respect to the coordinates of the vertex [48, 10].

When two vertices become too close and are about to intersect, we change the local connectivity of the tissue. To this end, a discrete event, known as T1 transition, is defined to resolve an edge that is about to vanish: a new edge is introduced,
Figure 3.2: Dorsal appendage formation during *Drosophila* oogenesis. (A) Schematic of cell types in the developing *Drosophila* egg chamber and the fully formed eggshell. Blue highlights the roof cells, red the floor cells, orange the midline cells and rest are main body cells. (B1-B5) 3D reconstructions of the apical surface of follicle cells at different time points during dorsal appendage formation in *D. melanogaster*. The flat primordium is transformed into a conical structure through a sequence of cell neighbor exchanges. The color-coding is same as before. Images are taken from [49], where it is described in greater detail.

perpendicular to the vanishing edge and passing through its mid-point. This event changes the local connectivity within the cell sheet (Fig. 3.1G). If the vertices are free to move in three-dimensions, cells adjacent to the shrinking edge can be non-coplanar. In such cases, an average plane is defined in which the T1 transition takes place (as described in greater detail in the Appendix B.6).
3.3 Modeling of dorsal appendage morphogenesis

In this section, we show how the proposed modeling framework can be used to describe morphogenesis of the respiratory appendages during Drosophila oogenesis, an established experimental model for studying the mechanisms by which patterned cell sheets give rise to complex three-dimensional structures.

The final product of Drosophila oogenesis is a single cell, the oocyte, surrounded by an elaborately patterned eggshell, a proteinaceous structure that houses the oocyte and the future embryo and mediates their interaction with the environment \[25\]. The eggshell plays a critical role in controlling the respiration of the embryo, ensuring adequate supply of oxygen and preventing dehydration. In a number of Drosophila species, including Drosophila melanogaster, the eggshell is adorned with respiratory appendages, which can vary in shape and number, and aid respiration when the egg is buried in a soft oviposition substrate, like a rotting fruit \[22, 71\]. The entire eggshell, including the respiratory appendages, is derived from the epithelial layer that surrounds the developing oocyte \[3\]. The apical surfaces of epithelial cells in this layer face the oocyte, while their basal surfaces adhere to a common extracellular matrix that surrounds the developing egg follicle. For several decades, the formation of dorsal appendages has served as an important model for genetic studies of tissue patterning and morphogenesis \[3, 52, 73, 16, 20\].

Most of our current understanding of this process has been derived from studies in Drosophila melanogaster, which has an eggshell with two respiratory appendages. Midway through oogenesis, the follicular epithelium is patterned by an inductive signal that establishes two groups of appendage-producing cells. Each group consists of two cell types a patch of roof cells that eventually form the top of the tube and a single-cell-wide row of floor cells that form the lower side of the tube (Fig. 3.2A). Each appendage primordium is made up of ~70 cells and is characterized by a specific pattern of increased apical contractility \[48\]. This pattern initiates a robust
morphogenetic transformation, in which the appendage primordium first bends out from the epithelium and is then transformed, through an ordered sequence of cell rearrangements, into a conical 3D structure (Fig. 3.2B1-B5, [48, 49]).

Both steps of this process have been recently simulated using a 2D vertex model [48]. In response to prepatterning, modeled through an increase in the energetic cost of areas and edges in a subset of cells, the primordium first buckles out of the sheet and then undergoes a stereotyped sequence of intercalations. Epithelial sheet was assumed to evolve in an over-damped setting. The computational models were based on the vertex description of epithelial sheets that were more complex than Eq. 3.1 but cannot capture the three-dimensional nature of the epithelium. Here we show how the model, with the added term describing the apicobasal polarity, can be used to describe the dynamics of appendage formation with minimal patterning.

This transformation relies on spatial and temporal patterning of mechanical properties of cells in the model epithelium. The first type of patterning increases the contractility of the roof cells, causing their uniform constriction and localized bending of the primordium in the direction away from the oocyte. In the model, this is implemented with the newly added term (Eqs. 3.3). This type of patterning is motivated by the observed increased levels of myosin in the roof cells [48]. The second type of patterning increases the line tension along the floor-midline boundary. This is realized through spatially varying the value of $\sigma$ in Eq. 3.1. Specifically, the value of $\sigma$ is peaked at the center of this boundary, motivated by the observed peaked localization of myosin in this region of the follicular epithelium (Fig. 3.3A,B,E). This type of patterning is also supported by experimental observations of myosin localization.

Computational analysis of the dynamics of a small primordium identified the tissue patterning, in terms of model parameters, needed for ordered cell rearrangements. A representative example is shown in Fig. 3.3 which demonstrates how the peaked line tension term at the floor/midline border induces repeated cell neighbor exchanges, in
Figure 3.3: Small appendage formation (the number of appendage-producing cells in the model is less than that present in the follicle cells in *Drosophila melanogaster*). (A) Initial flat configuration highlighting different cell types present during appendage formation. Yellow cells indicate midline cells, gray main body cells, red floor cells, and blue roof cells. (B) Schematic highlighting prepatterning of cell properties. The line tension coefficient for red edges $\sigma_{fm,0}$ (boundary of floor-midline cells) is larger than for the rest of the tissue. The contractile prepattern introduced to mimic apically constricting roof cells is shown in blue, for which $\Gamma_h \neq 0$. (C) Plot of the systems energy as a function of time, relative to the energy of the initial homogeneous configuration. A magnified portion of the same plot and the equilibrium configuration are shown inset. (Continued on next page)
which the line of the floor cells is progressively bent, as these cells lose their contacts with the midline cells. As a consequence of these cell rearrangements, the length of the interface between the floor and midline domains is decreased.

Our initial expectation was that these dynamics could be used to generate appendages from primordia independently of their size, as long as one dynamically re-centers the line tension at the midline/floor border. However, we found that the process in larger primordia fails and neighbor exchanges at the midline/floor border stall after the first few cell intercalations. We found that this problem can be repaired if the tissue outside of the appendage primordium is made more compliant, which can be implemented by decreasing the values of $\mu_s$ and $\sigma$ in Eq. 3.1 in all cells excluding the roof and floor domains. With this additional assumption, the system could form the conical structure with cell-cell connectivity that closely resembles the connectivity revealed by reconstruction of the experimental images of dorsal appendages (Fig. 3.4).

In summary, we used our computational model of multicellular dynamics to determine the minimal patterning strategy needed for the transformation of the flat primordium into a three-dimensional conical structure. This strategy combines uniform increase of cell contractility within the primordium, dynamically maintained and peaked profile of cell contractility along the border of the primordium, as well as uniform change of cell properties outside of the primordium. Our parametric studies
Figure 3.4: Model appendage formation (the number of appendage-producing cells in the model is similar to that present in the follicle cells in *Drosophila melanogaster*). (A) Initial flat configuration highlighting different cell types present during appendage formation. (B) Schematic highlighting prepatterning of cell properties. For red edges $\sigma = \sigma_{fm,0} > 1$; for grey edges $\sigma = 0.1$; otherwise $\sigma = 1$. For grey cells $\mu_s = 0.1$; otherwise $\mu_s = 1$. Offset contractile prepatterning is shown in blue ($\Gamma, h \neq 0$). (C1-C5) Sequence of representative shapes that emerge during the formation of the appendages after introducing position dependent value of $\sigma$ for red edges (as described in Fig. 3.3E). Figures on the left in the sub-panel highlight the floor+roof+midline cells. Figures on the right in the same sub-panel highlight the floor cells only (above) or floor+roof cells (below). Viewpoints are chosen to highlight the increase in floor-floor cells connectivity during the simulation. Parameter values are listed in Table E.4.

revealed that the resulting morphogenesis is robust with respect to reasonable variations in cell properties. Using the model, one can readily explore the differential contributions of the three different prepatterning mechanisms. In the future, some of the model predictions, such as the uniform change in the compliance of the tissue outside of the primordium, could be tested experimentally by direct measurement of cell-cell tensile forces. In parallel, one could computationally explore alternative mechanisms, such as directed migration of the roof cells, an effect that plays a key role during the later stages of dorsal appendage morphogenesis in *Drosophila melanogaster* [49, 51].
3.4 Discussion

Computational modeling of epithelial morphogenesis driven by cell shape changes and rearrangements requires models that can resolve individual cells. Current models of dynamic cell shapes in epithelial sheets are far behind the models of single cells, which can treat cell shapes using a free boundary value problem formulation \[8\]. Furthermore, describing some of the key aspects of cell dynamics in epithelial sheets, such as the emergence of wedge-like cell shapes in response to apical cell constriction, requires models that treat cells as three-dimensional objects \[39, 21\]. We believe that the presented model provides the simplest possible mathematical description that can simultaneously handle dynamic cell shape changes and mimics the apicobasal cell polarity in epithelial sheets. As was shown in our earlier study, the 2D model that ignores apical localization of contractile segments is not consistent with the complete three-dimensional description of an epithelial monolayer, especially bending due to the constriction of apical surface of a patch of cells. Addition of new terms that can effectively offset the contractile segments from the 2D sheet can capture the essential effects of the 3D model, while maintaining the simpler 2D framework \[39\]. Furthermore, the same model can be used to describe morphogenetic processes that involve ordered cell rearrangements.

Already at this point, this class of vertex model can be used to explore the feasibility of morphogenetic mechanisms proposed on the basis of live imaging studies of epithelial dynamics. For example, in the case of the respiratory appendage formation in *Drosophila melanogaster*, experiments suggested that the process is driven by the localized pattern of cell contractility that induces sequential neighbor exchanges leading to the formation of a conical structure \[48\]. Our computational exploration of this mechanism suggested that the localized pattern of contractility is not sufficient and must be accompanied by softening of the surrounding tissue. Without this added effect, the process stalls after just a few neighbor exchanges.
Chapter 4

Conclusions

In this thesis, we used vertex models to investigate how 2D prepatterns of apical contractility induce 3D deformations of epithelial sheets and proposed additional terms to the 2D vertex model framework to better capture 3D epithelial deformations. As discussed in section [1], a number of previous studies of such deformations used appropriately modified cell-based models to make a connection between the prepatterning and the bending of an epithelial sheet. The spatial patterns of apical contractility that drive 3D epithelial deformations are commonly fine grained. For instance, the early stages of sea urchin gastrulation are triggered by a contractile ring that is only two cells wide. These observations provide a clear motivation for developing models that can resolve individual cells.

We show that models of epithelia constructed from 3D cells, with distinct apical and basal properties, can generate both invaginated and evaginated states, depending on the apicobasal orientation of the epithelial shell. In the 3D model, constriction of the apical surface leads to the expansion of the basal surface due to the presence of the incompressible cytoplasm. Each cell now adopts a wedgelike shape that provides localized curvature to the epithelium and the epithelial monolayer bends towards the basal side. In contrast to the 3D vertex model, spatial patterns of mechanical
properties can induce shape bistability in naturally planar 2D vertex model. Two different stable steady states (invaginated and evaginated) co-exist for the same set of parameters. The emergence of this regime can be traced back to the pitchfork bifurcation in an axisymmetric flat setting, in which a contractile ring prepatter can induce buckling in an initially flat epithelial layer. Introducing the curvature breaks the symmetric pitchfork. As a consequence of this symmetry breaking, the evaginated state is obtained by continuous increase of the amplitude of the spatial pattern of cell contractility. The invaginated state exists as an isolated branch. However, in presence of a uniformly constricting patch, no out-of-plane deformations happen. This is in contrast to what is observed experimentally, where epithelial monolayer bends towards the basal direction due to a patch of apically constricting cells.

Motivated by capturing the essential features of the 3D model while maintaining the computational simplicity of the 2D model framework, we proposed a modified 2D model. We found that the 3D nature of an epithelial monolayer can be captured by adding two new terms to the energy function in the model with polygonal cells. We modeled cell sheet as the midsurface of an epithelial monolayer. The modified bending energy term can capture the natural curvature of the epithelial sheet and the additional prepatterning term mimics the effect of the contractile segments that are offset from the midsurface, reflecting the cell contractility on the apical side of the epithelium. With the addition of both these terms, the modified 2D model can capture the essential effects of the 3D model. In particular, the 2D model can generate both evaginated and invaginated shapes depending on the apical-basal polarity of the epithelial shell.

We also showed that the proposed modeling framework can be used to describe morphogenesis in more complex shape transformations which is driven by both bending as well as cell rearrangements. The model system is the formation of the respiratory appendages during *Drosophila* oogenesis, an established experimental model
for studying the mechanisms by which patterned cell sheets give rise to complex three-dimensional structures.

Epithelial morphogenesis accompanied by cell rearrangements can be viewed from two perspectives, kinematic and dynamic. First, the transformation between the initial and final structures, such as the flat primordium and fully formed appendage in our example, can be described by specifying an ordered sequence of neighbor exchanges. Second, one can ask about the forces that realize this sequence. In our work, the dynamic mechanism is suggested by the spatial patterns of myosin localization and computational modeling. Importantly, the question about the kinematics of transitions between the two states with different cell adjacencies can be viewed separately. When the number of cells in the system is constant, the full lists of cell adjacencies in the initial and final configurations can be represented as graphs and one can ask whether two different graphs can be connected by a path, in which each step is realized by a single T1 neighbor exchange. Formalizing this reachability problem and finding a way to solve it efficiently can provide insights into the mechanisms by which flat cell sheets can give rise to a wide range of three-dimensional structures. This purely kinematic approach may be especially useful in the analysis of experimental systems that are not readily amenable to live imaging. Also, when this kinematic path has been identified, the next step could be to use our model and identify a pattern of our model parameters that can drive the system along this path.

Although the present work emphasized mechanisms that rely on ordered cell rearrangements, one should keep in mind that real epithelial sheets can rely on strategies other than T1 exchanges. As an example, recent analysis of eggshell patterning in *Scaptodrosophila pattersoni* revealed that epithelial morphogenesis in this system relies not on ordered cell rearrangements, but on dramatic cell deformations, whereby the lengths of some cell edges become greatly elongated \[49\]. A modeling framework for describing these changes is yet to be developed. Overall, we conclude that the
proposed model can be used to explore multiple aspects of three-dimensional mor-
phogenesis of patterned cell sheets. However more work is required for making the
model more grounded in the biophysical description of epithelial tissues. As a step
in this direction, one may start with models composed of three-dimensional cells and
attempt to systematically reduce them to the models similar to the one described in
this thesis.
Appendix A

3D Model

A.1 Surface area, cell volume and inner-cavity volume

3D cells are modeled as ‘lampshade’ shaped prisms (Fig. A.1(A)). Given the coordinates and specific ordering of vertices (which we will explain more later), we can define edges, surfaces, and the volume of the cell. In this section, we will describe how we define each of these geometric features for numerical implementation.

The length of an edge, as formed by two neighboring vertices \( \mathbf{r}_{i+1} \) and \( \mathbf{r}_i \), is

\[
l_e = |\mathbf{r}_{i+1} - \mathbf{r}_i|,
\]

(A.1)

where \( \mathbf{r}_i = x_i \mathbf{i} + y_i \mathbf{j} + z_i \mathbf{k} \).

The nodes that constitute a ‘surface’ \( (\mathbf{r}_0, \mathbf{r}_1, ..., \mathbf{r}_{n-1} \) where \( n \) is the number of vertices in a surface) can be nonplanar. We build a surface from these nodes by defining triangular sub-elements (as formed by \( \mathbf{r}_i, \mathbf{r}_{i+1} \) and \( \mathbf{C}_{s1} \) and for \( i \in [0, n-1] \), as shown in Fig. A.1(B)). \( \mathbf{C}_{s1} \) is the centroid of the surface, which is defined as mean
Figure A.1: 3D Vertex Model. (A) Schematic representation of a ‘lampshade’ shaped 3D cell with distinct apical, basal, and lateral surfaces. (B) Schematic of a representative surface of a 3D cell showing triangular sub-elements as formed by surface nodes and surface centroid $C_{s1}$. The surface area is defined as the sum of the areas of triangular sub-elements (discussed more in [A.1]). (C) Schematic illustrating the \textit{apical-out} case, where the apical side forms the \textit{outer} surface of the epithelial shell. Apical constriction of a patch of cells leads to invagination. (D) Schematic illustrating the \textit{apical-in} case, where the apical side forms the \textit{inner} surface of the epithelial shell. Apical constriction of a patch of cells leads to evagination.

The area of the surface $A_{s1}$ is defined as the sum of the areas of triangular sub-elements in the following way

\[
A_{s1} = \sum_{i=0}^{n-1} |A_i|,
\]

\[
A_i = \frac{1}{2} (\mathbf{r}_{i+1} - C_{s1}) \times (\mathbf{r}_i - C_{s1}),
\]

\[
C_{s1} = \frac{1}{n} \sum_{i=0}^{n-1} \mathbf{r}_i.
\]
We define the ordering of the vertices such that the surface area vector (as defined in Eq. A.2) points in the ‘outward’ direction with respect to the cell. The volume of a cell is defined in the following way

\[
V_c = \sum_{j=0}^{n_s-1} V_j, \\
V_j = \sum_{i=0}^{n-1} \frac{1}{3} A_i \cdot C_j,
\]  

(A.3)

where \(n_s\) is the number of surfaces in the cell, and \(V_j\) is the (signed) volume of a pyramids formed by triangular sub-elements of the surface \(j\) as the base and the reference origin as the apex. The volume of the cell \(V_c\) can be calculated by taking the sum of the (signed) volume \(V_j\) over all surfaces of the cell. \(A_i\) is the area vector associated with the triangular sub element \(i\), and \(C_j\) is the centroid of surface \(j\) (as defined in Eq. A.2).

We use similar ideas to calculate the volume of the inner cavity of the epithelial shell \(V_Y\) and is defined in the following way

\[
V_Y = \sum_{k=0}^{N_b-1} V_k, \\
V_k = \sum_{i=0}^{n-1} \frac{1}{3} A_i \cdot C_k,
\]  

(A.4)

where \(N_b\) is the number of cell surfaces on the inner side of the shell. Note that the inner surface of the epithelial shell is formed by the apical side in the apical-in case and by the basal side in the apical-out case (see chapter 2 for details).
A.2 Numerical implementation of the energy gradient

To find equilibrium shapes, we find configurations with zero net force. In this section, we will describe how we found analytical expressions for forces on each vertex. The net force on a vertex \( r_i \) is given as,

\[
\mathbf{F}_i = -\nabla_i E = -\left( \frac{\partial E}{\partial x_i} \hat{i} + \frac{\partial E}{\partial y_i} \hat{j} + \frac{\partial E}{\partial z_i} \hat{k} \right).
\]  

(A.5)

This net force can be split into different terms corresponding to different energy contributions due to apical edges, lateral surfaces, basal surfaces, and due to the volume incompressibility term (eq. 2.1).

The force on a vertex \( r_i \) due to an apical edge \( e_1 \) is

\[
E_{e_1} = \sigma l_{e_1} = \sigma |r_{i+1} - r_i|,
\]

\[
F_{e_1} = -\sigma \frac{r_{i+1} - r_i}{|r_{i+1} - r_i|}.
\]  

(A.6)

We get the net force due to apical edges on a given vertex by adding the contribution from all three adjacent apical edges.

The force on a vertex \( r_i \) due to a lateral surface \( s_1 \) is

\[
E_{s_1} = \alpha A_{s_1},
\]

\[
F_{s_1} = -\alpha \nabla_i A_{s_1}.
\]  

(A.7)

Neighboring vertices of \( r_i \) in a surface \( s_1 \) (as defined before) are \( r_{i+1} \) and \( r_{i-1} \). We identify two triangular sub-elements \( A_1 \) and \( A_2 \) which have contributions from \( r_i \).
\[
A_{s1} = |A_1| + |A_2| + \sum_{j=0}^{n-1} |A_j|,
\]  
(A.8)

where \(A_1 = \frac{1}{2}(r_i - C_{s1}) \times (r_{i-1} - C_{s1})\), \(A_2 = \frac{1}{2}(r_{i+1} - C_{s1}) \times (r_i - C_{s1})\), and \(A_j = \frac{1}{2}(r_j - C_{s1}) \times (r_{j-1} - C_{s1})\).

Next, we show the terms involved in the definition of the derivative of the surface area that are needed to calculate the surface force.

\[
\nabla_i A_{s1} = \nabla_i |A_1| + \nabla_i |A_2| + \sum_{j=0}^{n-1} \nabla_i |A_j|,
\]

(A.9)

Using the definition for the area (Eq. A.2) and simple algebraic manipulations, we get the expressions for the terms involved in the derivative of the surface area (Eq. A.9 listed in Table A.1). Using these expressions, we can calculate the force due to the lateral surface \(s1\) (Eq. A.7). We use similar ideas to calculate the force on a given node due to the basal surface. We add the contributions from neighboring lateral and basal surfaces to find the net force on the vertex \(r_i\).
Table A.1: Expressions for derivatives of surface area terms involved in calculation of force terms (Eq. A.9). \( n \) is the number of vertices in a surface and the surface centroid is denoted by \( C_s = x_c \hat{i} + y_c \hat{j} + z_c \hat{k} \). See section A.2 for more details.

We use a similar approach to find the cell volume incompressibility force term. There are three neighboring cells for vertex \( r_i \). For one cell \( v_1 \) containing vertex \( r_i \), we calculate the derivative in the following way

\[
E_{v1} = B(V_{v1} - V_0)^2, \\
F_{v1} = \nabla_i E_v = 2B(V_{v1} - V_0)\nabla_i V_{v1}, \\
\nabla_i V_{v1} = \sum_{j=0}^{n_s-1} \nabla_i V_j.
\]
\[ \frac{\partial V_j}{\partial x_i} = \frac{1}{3} \sum_{i=0}^{n-1} \left( \left( \frac{\partial A^x_i}{\partial x_i} \hat{i} + \frac{\partial A^y_i}{\partial x_i} \hat{j} + \frac{\partial A^z_i}{\partial x_i} \hat{k} \right) \cdot C_j + \frac{1}{n} A^z_i \right), \]

\[ \frac{\partial V_j}{\partial y_i} = \frac{1}{3} \sum_{i=0}^{n-1} \left( \left( \frac{\partial A^x_i}{\partial y_i} \hat{i} + \frac{\partial A^y_i}{\partial y_i} \hat{j} + \frac{\partial A^z_i}{\partial y_i} \hat{k} \right) \cdot C_j + \frac{1}{n} A^y_i \right), \]

\[ \frac{\partial V_j}{\partial z_i} = \frac{1}{3} \sum_{i=0}^{n-1} \left( \left( \frac{\partial A^x_i}{\partial z_i} \hat{i} + \frac{\partial A^y_i}{\partial z_i} \hat{j} + \frac{\partial A^z_i}{\partial z_i} \hat{k} \right) \cdot C_j + \frac{1}{n} A^z_i \right). \]

We already have the expressions for the surface area derivative terms (Table A.1) needed to calculate the cell volume incompressibility force term. The approach is extended to find the expression for force corresponding to the internal fluid and to the outer membrane term.

### A.3 Stable homogeneous configuration for the 3D vertex model

To obtain a good guess for the geometric and material properties for a stable homogeneous shell, we minimize the energy of a single cell in ideal cases. We consider two cases: hexagonal prism-shaped and lampshade-shaped cells (Fig. A.2(A,B)). Hexagonal prism-shaped cells build a flat sheet whereas lampshade-shaped cells build a curved sheet.

In the case of hexagonal prism-shaped cells, both the basal and apical surfaces are represented by equivalent hexagons with edge length \( a \) and height \( h \). We can write the non-dimensionalized form of the energy for a single cell by scaling length by \( V_0^{1/3} \) and energy by \( \sigma V_0^{1/3} \). We obtain the following equation for a hexagonal prism-shaped cell with three re-scaled parameters (keeping similar nomenclature after renaming : \( \frac{aV_0^{1/3}}{\sigma} \equiv \alpha, \frac{\gamma V_0^{1/3}}{\sigma} \equiv \gamma, \frac{BV_0^{5/3}}{\sigma} \equiv B \)).

\[ E_c = 3a + 3aah + \gamma \frac{3\sqrt{3}}{2} a^2 + B \left( \frac{3\sqrt{3}}{2} a^2 h - 1 \right)^2 \]  

(A.10)
Figure A.2: Cell shape as a function of model parameters for a hexagonal prism shaped and a lampshade shaped cell. (A) Hexagonal prism, part of a flat sheet, with height $h$ and hexagonal edge $a$. (B) Lampshade-shaped prism, part of a curved sheet, with height $h$, apical edge $a_1$ and basal edge $a_2$. (C) The minimal energy states of a hexagonal prism shaped cell as a function of basal surface energy coefficient $\gamma$ for a given lateral surface energy coefficient $\alpha$. (D) The minimal energy states of a lampshade-shaped cell as a function of basal surface energy coefficient $\gamma$ for a fixed basal edge length $a_2$. Schematics of the minimum energy configurations are shown as insets.

If we take the cell volume compression modulus to be large ($B \to \infty$), we can assume that $V_C$ (cell volume) remains similar to $V_0$ (initial cell volume). This assumption provides a relationship between two geometric variables of the hexagonal prism, $a$ and $h$ ($h = \frac{2}{3\sqrt{3}a^2}$). The non-dimensionalized energy equation for a single cell reduces to

$$E_c = 3a + 3\alpha \frac{2}{3\sqrt{3}a} + \gamma \frac{3\sqrt{3}}{2} a^2 \quad \text{(A.11)}$$

The minima of the energy function, ($\frac{dE_c}{da} = 0$, $\frac{d^2E_c}{da^2} > 0$) give a relationship between the material properties ($\alpha, \gamma$) and the geometric parameter ($a$) that we use to generate an initial flat homogeneous configuration (Fig. A.2(C)).
For lampshade-shaped cells, the edge lengths of the apical hexagonal face \( a_1 \) and the basal hexagonal base \( a_2 \) are different \( (V_c = \frac{\sqrt{3}}{2}(a_1^2 + a_2^2 + a_1 a_2)h) \). Following the previous analysis, the reduced energy expression for a lampshade-shaped cell is given by the following expression

\[
E_c = 3a_1 + 3\alpha \frac{a_1 + a_2}{\sqrt{3(a_1^2 + a_2^2 + a_1 a_2)}} + \gamma \frac{3\sqrt{3}}{2} a_2^2
\] (A.12)

This is a two variable minimization problem. The minima of this function \( (\frac{\partial E_c}{\partial a_1} = 0, \frac{\partial E_c}{\partial a_2} = 0 \) and positive definite Hessian matrix) give a relationship between the material properties and the geometric parameters for the stable homogeneous shell (Fig. A.2(D)).

In Fig. A.2(C,D), we plot stable states for a single cell (solution for the minimization problem) around parameter values that we have used for the numerical experiments. In the case of hexagonal prism-shaped cells, if we decrease \( \gamma \) at fixed \( \alpha \), cells become squamous (smaller \( h \)). For lampshade-shaped cells with fixed basal surface edge length \( (a_2 = 0.5) \), increasing \( \gamma \) inverts the curvature. Above the value of \( \gamma \) for which cells are flat \( (a_1 = a_2) \), the apical side becomes the inside of the shell \( (a_1 > a_2) \), while below that value of \( \gamma \) the apical side becomes the outside of the shell \( (a_1 < a_2) \). Hannezo et al. did a similar analysis for their 3D model [21].

### A.4 Effect of model parameters on deformations

We used the non-dimensionalized energy equation \( (V_0 = 1, \sigma = 1) \) for the following analysis. We kept \( \alpha = 4 \) fixed and the values of \( \gamma \) for which different aspect ratios of the homogeneous sheet (made up of hexagonal prisms) were at equilibrium and used them as the initial conditions. We considered three different cases here, \( a = 0.5, \gamma = 5.96, a = 0.7, \gamma = 1.77 \) and \( a = 0.8, \gamma = 0.58 \). Other parameters used...
were $\sigma = 1, B = 100$. We observed more deflection for the same force heterogeneity for thinner sheets, while the qualitative nature of the steady state diagram remains similar (Fig. A.3).

We compared equilibrium shapes of homogeneous shell ($\Gamma = 0$) in the presence and absence of the outer membrane term for both cases: apical-in (Fig. A.4(A-B)) and apical-out (Fig. A.4(C-D)). Similar to the naturally planar 2D vertex model, we observed that the outer membrane term kept the closed surface more spherical. We also assessed the effect of inner-fluid compression modulus ($B_Y$) for both the apical-in (Fig. A.5(C,D)) and apical-out (Fig. A.5(A,B)) cases. If we increased $B_Y$, keeping other parameters constant, we saw a decrease in the deflection (Fig. A.5).

![Figure A.3: Effect of cell aspect ratio on bending for the 3D vertex model due to an apically embedded contractile ring. (A-C) Initial configurations with edge length 0.5, 0.7 and 0.8, respectively. Red edges $\Gamma > 0$; other edges $\Gamma = 0$. (A'-C') Cross-sections of invaginated steady states. (D) Steady state diagram showing deflection $\delta$ with increasing $\Gamma$. Parameter values are discussed in the Section A.4.](image)

**A.5 Effect of curvature on deformations**

We analyzed the effect of prepatterning and curvature on the equilibrium shapes of epithelial shells made up of 3D cells. We considered shells of four different curvatures...
Figure A.4: Effect of the outer membrane term for the 3D vertex model. (A,B) Initial homogeneous stable configuration for the *apical-in* case (see main text for details). (A) In the presence of the outer membrane term ($\epsilon = 10^{-10}$). (B) In the absence of the outer membrane term ($\epsilon = 0$). (C,D) Initial homogeneous stable configuration for the *apical-out* case (see main text for details). (C) In the presence of the outer membrane term ($\epsilon = 10^{-10}$) (D) In the absence of the outer membrane term ($\epsilon = 0$). (E-H) Cross-section of (A-D) respectively. The shape is more spherical in the presence of the outer membrane term in both cases. Parameter values used and geometry of the epithelial shell are the same as in the main text analysis (Table E.1).

$C$ (= inverse of the radius of spherical shells from which the system is initialized, Fig. A.7(A)). We used epithelial cells with the following geometric and model parameters for all cases discussed here: $a_B = 0.55, h = 1.0, \gamma = 0.95, \alpha = 1.9, \sigma = 1$. The approximate nature of meshing on the spherical surface caused the number of cells (210 ± 10) and the basal edge length ($a_{basal} = 0.55 ± 0.01$) to be slightly different for different shell configurations (see section 2.6 for details). We kept the boundaries of the shells fixed for both apical and basal surfaces.

We found a single continuous branch of stable steady states for apical constriction of a patch of cells. In this case, deflection monotonically increases with increasing $\Gamma$ (Fig. A.7(G)). On the other hand, we observed bistability in the shapes of shells for the contractile ring prepattern in shells above a certain curvature (Fig. A.7(H)). There were two stable shapes for the same value of $\Gamma$, one with a slightly evaginating patch of cells (deflecting outward) and one with an invaginating patch of cells (deflecting...
Figure A.5: The effect of the inner fluid incompressibility term on deflection for the 3D vertex model. (A-D) Steady state diagrams showing deflection $\delta$ for different heterogeneities. (A) Contractile ring for the apical-out case. (B) Patch of apically constricting cells for the apical-out case. (C) Contractile ring for the apical-in case. (D) Patch of apically constricting cells for the apical-in case. Black curve: $B_Y = 0$; Brown curve: $B_Y = 0.001$. Solid (dashed) line: stable (unstable) steady states. Schematic cross-sections of representative states are shown as insets. Deflection is typically less for increased inner-fluid compression modulus. Parameter values used and geometry of the epithelial shell are same as main text analysis (Table E.1).

inward). The upper limit point in the branch of steady states defined a threshold for the heterogeneity parameter, after which we observed a sudden bending of the sheet.
Figure A.6: 3D deformations induced by a ring of apically constricting cells, one-cell wide, with 3D cells for the *apical-out* case. (A) Initial configuration showing the outer surface (i.e. apical side) of the shell. (B) Representative invaginated state. (C) Steady state diagram showing deflection $\delta$ with increasing parameter $\Gamma$. Cross-sections of representative steady states (A,B) are shown as insets. Parameter values are listed in Table E.1 (similar to Fig. 2.3).
Figure A.7: The effect of curvature on bending for the 3D vertex model. (A) Four different initial configurations with curvatures $C = 0.2, 0.15, 0.1, \text{ and } 0$, respectively. (B) Contractile ring heterogeneity, highlighted on the initial configuration with $C=0.2$. (C) Constricting patch heterogeneity, highlighted on the initial configuration with $C=0.2$. The number of cells enclosed by the cable or included in the patch were similar for all initial curvatures. (D,E) Cross-sections of B and C respectively. (F-G) Steady state diagram showing deflection $\delta$ with increasing parameter $\Gamma$. Solid (dashed) line: stable (unstable) steady states. Color-coding of curves according to initial configuration matches color coding in A. Cross sections of representative steady states are shown as insets. Parameter values are listed in Section A.5.
Appendix B

2D Model

B.1 Bending energy term with natural curvature

Figure B.1: 2D vertex model. (A) Schematic to explain offset contractile segment (shown in red) in the 2D vertex model. The figure shows two adjacent cells $S_1$ and $S_2$ with shared edge $j$, centers ($C_{S1}$, $C_{S2}$) and unit normals ($N_{S1}$, $N_{S2}$). $u_j$ is the unit vector joining the cell centers. The red line highlights a contractile segment joining the cell centers that is offset by a distance $h$ from the surface along the cell normals. (B) Schematic of the configuration shown in (A) as viewed in the plane perpendicular to the common edge. Here, for the sake of illustration, we are assuming that each of the surface is planar or very close to planar.

The naturally planar 2D vertex model has a bending energy term which corresponds to a naturally flat sheet. We modify it to represent natural curvature of the sheet in the following way.
\[ E_{\text{bend}} = \beta \sum_{j'} \left( (1 - \mathbf{N}_{S2(j')} \cdot \mathbf{N}_{S1(j')}) - k_{j'} \left(- (\mathbf{N}_{S2(j')} - \mathbf{N}_{S1(j')}) \cdot \mathbf{u}_{j'} \right) \right). \quad \text{(B.1)} \]

The modified bending energy term defines an equilibrium value of the angle between the adjacent normals of the interior edges. We define the normal of the cell \( S \) in the following way

\[
\mathbf{N}_S = \frac{\mathbf{A}_S}{|\mathbf{A}_S|},
\]

\[
\mathbf{A}_S = \frac{1}{2} \sum_{i=0}^{n_v-1} (\mathbf{r}_i \times \mathbf{r}_{i+1}),
\]

where \( n_v \) is the number of vertices of the cell \( S \). List of vertices are ordered in each cell such that the normal vector points ‘outside’ the shell (Fig. B.1, for further detail about the implementation of the 2D model refer to [41]).

Consider the two cells \( S1 \) and \( S2 \) adjacent to a particular interior edge \( j' \) (Fig. B.1). Suppose that the normals \( \mathbf{N}_{S1} \) and \( \mathbf{N}_{S2} \) stay coplanar with the unit vector \( \mathbf{u}_j \), as depicted in Fig. B.1B. Let \( \theta \) be the angle between the normals \( \mathbf{N}_{S1} \) and \( \mathbf{N}_{S2} \) and assume that the unit vector \( \mathbf{u}_j \) is perpendicular to the average normal i.e. \( \mathbf{N}_{S1} \) and \( \mathbf{N}_{S2} \) make an angle \((\pi - \theta)/2\) and \((\pi + \theta)/2\) with \( \mathbf{u}_j \), respectively. In the modified bending energy term (eq. B.1), the contribution coming from this segment is proportional to

\[
1 - \cos \theta - k_{j'}(-2 \sin(\theta/2)). \quad \text{(B.2)}
\]

The new term in the bending energy defines a rest value (i.e when the configuration is at equilibrium) of the angle between adjacent normals. This implements the notion of natural curvature precisely. Indeed, minimization of the bending energy in equation
above with respect to theta yields the rest value $\theta_{eq}$ of the angle $\theta$ as a function of $k_{j'}$. In
the particular case $|\theta| \ll 1$, the bending energy contribution can be approximated as

$$\frac{\theta^2}{2} + k_{j'} \theta \approx \frac{(\theta + k_{j'})^2}{2} - \frac{k_{j'}}{2}, \quad (B.3)$$

and the rest value of the angle between adjacent normals is given by $\theta_{eq} = -k_{j'}$.

If $k_{j'}$ is constant for all segments, the shell has homogeneous natural curvature. If
$k_{j'} = 0$, bending energy corresponds to a naturally flat sheet. For the homogeneous
shell configuration analysis, we set $k_{j'}$ as $l_c/R$ for all the edges where $R$ is the radius of
the initial spherical configuration and $l_c$ is the approximate average distance between
the cell centers ($l_c = 2\pi \cos(\pi/6)$ where $\pi$ is the average edge length of the polygonal
cells). As an example, for the parameters listed in Table E.3 (with $k_{j'} = 0.19$),
the angle between normals for the adjacent cells for the closed shell homogeneous
equilibrium configuration (i.e. with no prepatterning) is $\theta = 0.19 \pm 0.02$, which is
very close to the value of $k_{j'}$.

\section*{B.2 Offset contractile segments}

In the new implementation, contractile segments are introduced along the cell centers
and the additional energy of the prepatterning is defined as $E_\Gamma$ (Eq. 2.5). $E_\Gamma$ is the
linear truncation of $\tilde{E}_\Gamma$. We define $\tilde{E}_\Gamma$ in the following way,

$$\tilde{E}_\Gamma = \Gamma \sum_{j=1}^{n} \left( |(C_{S1(j)} + hN_{S1(j)}) - (C_{S2(j)} + hN_{S2(j)})| \right). \quad (B.4)$$

Essentially, the offset contractile segments are the edges joining the points $(C_{S(j)} + \ hN_{S(j)})$. The vertices of this segment are obtained by offsetting the cell centers using
the normals with an offset length $h$ (Fig. B.1). This term does two things: it applies
a line tension that tries to bring the cell centers close together; additionally, when
\( h \neq 0 \), it tries to bend the surface so that the endpoints of the normal vectors move closer together \((h > 0)\) or further apart \((h < 0)\), in such a way that the offset segment is as short as possible. The second effect changes the rest angle between the normals of the cells joined by the contractile segment. This term is similar to that of the modified bending energy term (Eq. B.1), as both induce a local change in the natural curvature of the shell.

B.3 Effect of curvature on deformation - Naturally planar 2D vertex model

We introduced a contractile ring (shown with red edges in Fig. B.2(A) where \( \Gamma > 0 \)) in the flat (Fig. B.2(A)) and curved (Fig. B.2(B)) configurations. Similar to Murisic et al. [41], we observed a supercritical pitchfork bifurcation as we increased \( \Gamma \) for the flat configuration with fixed boundary vertices (Fig. B.2(D)). For the slightly curved configuration, we found a typical imperfect pitchfork bifurcation. Solutions with a patch of cells deflecting outward (evaginated state) formed a continuous branch of steady states from an initially homogeneous configuration, whereas solutions with a patch of cells deflecting inward (invaginated state) formed an isolated branch (Fig. B.2(D)). Parameter values used for the simulation are \( \mu = 2.45, \sigma = 1, \beta = 0, N_C = 217 \) and \( \pi = 0.55 \).

B.4 Effect of model parameters on deformations - Naturally planar 2D vertex model

We compared the equilibrium shapes of homogeneous shell \((\Gamma = 0)\) in the presence and absence of the outer membrane term (Fig. B.3(A,B)). We found out that the outer membrane term kept the closed surface more spherical. We also assessed the
Figure B.2: The effect of curvature on bending of the naturally planar 2D vertex model. (A) Flat homogeneous equilibrium configuration, showing a contractile ring prepattern. (B) Curved homogeneous configuration, which is a part of a shell (with radius = 20). (C) Representative equilibrium configuration for the flat configuration with $\Gamma > 0$ (where the enclosed patch is bending ‘outward’). (D) Steady state diagram showing deflection $\delta$ with increasing parameter $\Gamma$ for flat (black curve) and curved (brown curve) initial configuration. Solid (dashed) line: stable (unstable) steady states. Curvature results in an imperfect pitchfork bifurcation. Schematic cross-sections of representative states are shown as insets. Parameter values are listed in section B.3.

Effect of inner-fluid compression modulus ($B_Y$) and bending elasticity coefficient ($\beta$) for the naturally planar 2D vertex model. Increasing $B_Y$ or $\beta$, while keeping the other parameters constant, increased the threshold after which invaginated states arises for the contractile ring prepatterning (Fig. B.3(D,E)).

**B.5 Effect of model parameters on deformations - 2D vertex model**

Here, we first analyze the effect of the imposed prepatterning, either in the form of a contractile ring or a constricting patch for different model parameters. Segments
Figure B.3: Effect of the outer membrane term and inner fluid incompressibility term on bending for the naturally planar 2D vertex model. (A,B) Initial homogeneous stable configuration: (A) In the presence of the outer membrane term ($\epsilon = 10^{-10}$); (B) In the absence of the outer membrane term ($\epsilon = 0$). The shape is more spherical in presence of the outer membrane term. Parameters used for the simulation are $\mu = 2.26$, $R_c = 5.025$, $n = 4$, $\sigma = 1$, $B_Y = 0$, $\beta = 0$ and the geometric parameters of the shell are same as main text analysis. (C) Steady state diagram showing deflection $\delta$ with increasing parameter $\Gamma$. $\Gamma_{cr}$ is the limit point after which invaginated equilibrium shapes arises. (D,E) Region of bistability for the parameters: (D) bending elasticity coefficient ($\beta$) (for fixed $B_Y = 0.001$) and (E) Inner-fluid compression modulus ($B_Y$) (for fixed $\beta = 0$). Other parameters are same as (A).
Figure B.4: 3D deformations induced by symmetric prepatterns of line tension. (A) Initial configuration highlighting a contractile ring prepattern. (B) Representative stable equilibrium configuration, where the enclosed patch bends outward (i.e. $\delta > 0$, $\delta$ is defined as the out-of-plane deflection of the mean of the node coordinates corresponding to the central cell). (C) Representative stable equilibrium configuration, where the enclosed patch bends outward (i.e. $\delta > 0$). (D) Initial configuration highlighting a constricting patch prepattern. (E) Representative equilibrium configuration with zero offset parameter ($h = 0, \Gamma \neq 0$). (F) Representative equilibrium configuration, where the enclosed patch bends inward (i.e. $\delta < 0$). (G) Steady state diagram showing deflection $\delta$ with increasing parameter $\Gamma$ for a contractile ring prepattern. Cross-sections of representative steady states (A-C) are shown as insets. (H) Steady state diagram showing deflection $\delta$ with increasing parameter $\Gamma$ for a constricting patch prepattern. Cross-sections of representative steady states (D-F) are shown as insets. Solid (dashed) line: stable (unstable) steady states. Parameter values are listed in table E.5.
belonging to either type of prepattern are assigned a line tension $\Gamma$ and an offset parameter $h$ (eq. 2.2). We start with a flat homogeneous configuration and increase the value of $\Gamma$ for prepatterned segments for different values of $h$ (highlighted in red in Fig B.4A and B.4D).

For a symmetric contractile ring case, with no offset (i.e. $h = 0$), we observe a pitchfork bifurcation (Fig. B.4G). After a certain threshold in $\Gamma$, flat configuration becomes unstable and pair of symmetric buckled shapes appears (Fig. B.4G). This is consistent with the pitchfork bifurcation observed by Murisic et al. (15) in response to the contractile ring prepattern. For $h \neq 0$, we find two disconnected branches of steady state solutions (Fig B.4G). We observe that the turning point of the isolated branch shifts to the right as we increase the value of $h$. For a symmetric constricting patch case, we find one continuous branch of equilibrium shapes as we increased $\Gamma$ (Fig B.4H). For $h = 0$, we do not observe any out of plane deformation. As we increase $h$, we get larger deflection for the same value of $\Gamma$.

Similar to the 3D vertex model, we observed that increasing inner-fluid compression modulus $B_Y$, while keeping other parameters constant, caused a decrease in the deflection for both apical-in and apical-out case (Fig. B.5).

### B.6 Defining T1 transition for non-planar cells

Epithelial sheet is assumed to evolve in an over-damped setting. This leads to the dynamics of the vertex $i$, with position vector $x_i$, governed by the following equation

$$\frac{d x_i}{dt} = \frac{\eta}{\eta_d} F_i = -\nabla E,$$

(B.5)

where $\eta$ is the mobility coefficient and $F_i$ is the force acting on the vertex $i$.

Keeping this in mind, we define a threshold length $L_t = 2\sigma_e dt/\eta$, where $dt$ is the time step, $e$ is a particular edge, $\sigma_e$ is the corresponding line tension coefficient and $\eta$
Figure B.5: Effect of the inner fluid incompressibility term on bending for the 2D vertex model. (A-D) Steady state diagrams showing deflection \( \delta \) for different heterogeneities. (A) Contractile ring for the apical-out case. (B) Patch of constricting cells for the apical-out case. (C) Contractile ring patterning for the apical-in case. (D) Patch of constricting cells for the apical-in case. Black curve: \( B_Y = 0 \); Brown curve: \( B_Y = 0.001 \). Solid (dashed) line: stable (unstable) steady states. Schematic cross-sections of representative states are shown as insets. Deflection is less for increased inner-fluid compression modulus. Parameter values used and geometry of the epithelial shell are same as main text analysis (Table E.3).

Figure B.6: Schematic of a T1 transition. Different terms shown in the figure are described in greater detail in section B.6.
is the mobility coefficient. This approximates the threshold length below which the edge will vanish in next time step. Our main assumption is that the velocity of both the vertices that comprises the edge is approximately equal to $\sigma/\eta$. If the length of the edge is below the threshold length, we implement a T1 transition event before the next time step that leads to cell neighbor exchange.

Let us say that the edge $e$, defined by two vertices $i_0$ and $i_1$, is shared by two adjacent cells $C_1$ and $C_2$ with corresponding unit normal vectors $N_1$ and $N_2$ (Fig. [B.6]). The unit normal vectors are defined in the following way

\[
A_1 = \frac{1}{2} \sum_{i=0}^{n-1} (r_i \times r_{i+1}), \quad (B.6)
\]

\[
N_1 = \frac{A_1}{|A_1|},
\]

where $n$ is the number of vertices in a cell and $r_i$ is the position vector of vertex $i$.

We then define a direction vector $u$ in the following way

\[
u = N_e \times (r_{i0} - r_{i1}), \quad (B.7)
\]

where $N_e$ is the average normal vector ($N_e = \frac{1}{2}(N_1 + N_2)$).

The direction vector is perpendicular to both the edge $e$ and the vector that defines the average plane of adjacent cells. New edge (with length = $1.1 \times L_t$) is defined along the vector $u$ with similar mid-point as the old edge $e$, followed by exchange of cell neighbors (Fig. [B.6]).
Appendix C

Initial configuration, continuation, and symmetry

In the main text, we discussed the construction of the initial configuration and the numerical methods we used to find the equilibrium configuration. Here, we give further details about the numerical aspects of the implementation, particularly on the role of the inherent translational and rotational symmetry of the system.

Using the approach described in Section A.3, we obtain the parameter values for which a shell made up of identical ideal cells are at equilibrium. Then, we use DistMesh [50], a meshing package that gives a triangular tessellation of a given edge length, to build a mesh on the sphere that is close to an ideal case configuration. We use the dual mesh, which is made up of the centers of the triangular elements, to construct polygonal cells. This forms the initial configuration for the 2D model and the inner surface of the epithelial shell for the 3D model. The vertices on the inner surface can then be extended radially to get an epithelial shell made up of 3D cells with distinct top and bottom surfaces. Here, we want to emphasize that not all apical and basal surfaces are hexagons in our initial spherical configuration - some are pentagons and heptagons. For example, in the case of a 2D initial spherical
configuration with edge length = 0.55 and radius = 5.0, we have 346 hexagons, 40 pentagons and 28 heptagons after meshing. Although configurations built using the meshing package differ slightly from the ideal cases, where all cells should have same morphology, they still provide a good initial guess for finding the homogeneous equilibrium configuration, which then serves as the initial point for the prepatternning analysis.

To implement the Newton-Raphson method and pseudo-arclength continuation, we approximate the derivatives of the forcing function i.e. the Jacobian (or equivalently the Hessian matrix in this case) by the first order forward finite-difference method (one could also use automatic differentiation). This complete Jacobian has six zero eigenvalues at a steady state for systems with rotational and translational symmetry, for example a closed shell in the absence of the outer shell term. We “pin” an appropriate number of vertices to make the Jacobian non-singular and to remove neutral stability directions (i.e. translationally and rotationally invariant solutions). We fix the coordinates of one vertex to be the origin, restrict the motion of a second vertex to only the radial direction, and restrict that of a third vertex to the radial and azimuthal directions to account for the six degrees of freedom. In the presence of the outer shell term, the system has only rotational symmetry, and pinning of a vertex is relaxed in such cases.
Appendix D

Definition of $\delta$

Figure D.1: Defining $\delta$ to quantify bending of an epithelial shell due to the prepatterning of the line tension. $C_0$ is the center of the initial homogeneous configuration with no prepatterning ($\Gamma = 0$). We use it as a reference center to define radial distance of the vertices. (A) A configuration with a constricting patch prepattern, highlighting cell vertices on the patch boundary (shown with red dots) and center of the patch (shown with a green dot). (B) For the 2D vertex model, $\delta$ is defined as the difference between the radial distance, from $C_0$, of the center of the patch ($R_c$) and the average radial distance of the vertices on the patch boundary ($\bar{R}_p$). (C) For the 3D vertex model, $\delta$ is defined as the difference between the radial distance of the center of the patch ($R_c$) and the average radial distance of the vertices on the patch boundary ($\bar{R}_p$) for the inner surface of the shell.
Appendix E

Parameters used in simulations

Table E.1: Parameter values used for simulations in section 2.7.1, where \( N_c \) is the total number of cells, \( \overline{a_A} \) is the average length of apical edges, \( h \) is the height of cells, \( \overline{a_B} \) is the average length of basal edges, \( R_A \) is the radius of the initial spherical configuration that makes the apical surface for the \textit{apical-in} case, and \( R_B \) is the radius of the initial spherical configuration that makes the basal surface for the \textit{apical-out} case. Other parameters are defined in corresponding main text.

<table>
<thead>
<tr>
<th>Figure 2.2: apical-in configuration</th>
<th>( \alpha = 2.13, \gamma = 0.98, \sigma = 1, B = 100, B_Y = 0, \epsilon = 10^{-10}, R_c = 6.025, n = 4 )</th>
<th>( N_c = 414, \overline{a_A} = 0.55, h = 1.0, R_A = 5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2.3: apical-out configuration</td>
<td>( \alpha = 1.89, \gamma = 0.94, \sigma = 1, B = 100, B_Y = 0, \epsilon = 10^{-10}, R_c = 6.025, n = 4 )</td>
<td>( N_c = 414, \overline{a_B} = 0.55, h = 1.0, R_B = 5 )</td>
</tr>
</tbody>
</table>

Table E.2: Parameter values used for simulations in section 2.7.2, where \( N_c \) is the total number of cells, \( a \) is the edge length of hexagons that constitute flat configurations, \( \overline{a} \) is the average edge length of polygonal cells that constitute curved configurations, \( R_i \) is the radius of the curved configuration, and \( R_0 \) is the radius of the initial spherical configuration. Other parameters are defined in corresponding main text.

| Figure 2.4: embedded contractile ring in a closed shell configuration | \( \mu = 2.26, \sigma = 1, \beta = 0.005, B_Y = 0, \epsilon = 10^{-10}, R_c = 5.025, n = 4 \) | \( N_c = 414, \overline{a} = 0.55, R_0 = 5 \) |
Table E.3: Parameter values used for simulations in section 2.7.3. Initial spherical configuration is same as previous analysis with the naturally planar 2D vertex model (Table E.2). $h, k_j'$ are defined in main text. Definition of other variables are similar to Table E.2.

Table E.4: Parameter values used for simulations in section 3.3. $N_c$ is the total number of cells, $a$ is the edge length of the regular hexagons used to construct the initial configuration. Other parameters are defined in corresponding main text.

Table E.5: Parameter values used for simulations in figure B.4. $N_c$ is the total number of cells, $a$ is the edge length of the regular hexagons used to construct the initial configuration. Other parameters are defined in corresponding text.
Bibliography


