Defect-mediated morphogenesis

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Growing experimental evidence indicates that topological defects could serve as organizing centers in the morphogenesis of tissues. In this article we provide a quantitative explanation for this phenomenon, rooted in the buckling theory of deformable active polar liquid crystals. Using a combination of linear stability analysis and computational fluid dynamics, we demonstrate that confined cell layers are unstable to the formation of protrusions in the presence of disclinations. The instability originates from an interplay between the focusing of the elastic forces, mediated by defects, and the renormalization of the system’s surface tension by the active flow. The post-transitional regime is also characterized by several complex morphodynamical processes, such as oscillatory deformations, droplet nucleation and active turbulence. Our findings offer an explanation of recent observations on tissue morphogenesis and shed light on the dynamics of active surfaces in general.

The development of multicellular organisms crucially hinges on internal and external mechanical cues which are transduced by the mechanosensory machinery of cells to give rise to system-wide spatiotemporal rearrangements and, eventually, to the formation of early embryonic features [1, 2]. These processes comprise a vast spectrum of active and passive forces, whose regulation relies not only on the molecular repertoire of tissue-forming cells, but, furthermore, on their shape, motility, and local organization.

One of the most fascinating hypotheses in this respect revolves around the possibility that multicellular systems could take advantage of topological mechanisms to create the conditions, in terms of reproducibility and robustness, for the origin and maintenance of life. In particular, defects have recently been identified by several in vitro studies as potential candidates for the role of “topological morphogens” in various biomimetic systems and model organisms. Keber et al., for instance, demonstrated that protocells, consisting of a single layer of microtubule-kinesin active nematic liquid crystal enclosed in a lipid vesicle and powered by adenosine triphosphate (ATP), relieve the mechanical stress, originating from the presence of four topologically required +1/2 disclinations, by growing persistent tubular protrusions (Fig. 1a and Ref. [8]). More recently, the same mechanism has been invoked in experimental [9–12] and theoretical [4, 13, 14] studies to explain the growth and regeneration of tentacles in Hydra (Fig. 1b). The accumulation of extensile stresses in proximity of integer and semi-integer disclinations has also been proposed by Saw et al. as a strategy to achieve cell apoptosis and extrusion in epithelial layers [15] and, more recently, by Guillamat et al. as a route to the formation of multicellular protrusions in confined myoblasts (Fig. 1c and Ref. [16]). Long before the importance of defects for the development of non-planar features in tissues had been recognized, blister-like hemicysts – also known as “domes” – were already routinely observed in many epithelial cell cultures derived from renal cell lines (see e.g. Ref. [6, 17, 18]). An example of a dome, obtained from a monolayer of epithelial Madin-Darby Canine Kidney (MDCK) cells, was reproduced here and is shown in Fig. 1d (see also Movie S1 and S2 and Fig. S1). Analogously to the previously illustrated examples, the central region of the dome, where the curvature is larger than elsewhere, features a multitude of topological defects (i.e. 5− and 7−fold cells) with net positive topological charge: i.e. \( \sum_{i \in \text{dome}} (6 - c_i) > 0 \), with \( c_i \) the number sides of the \( i \)-th cell.

Whereas these insightful experimental studies have now convincingly proved the existence of a correlation between topological defects and morphological features, especially dome- and protrusion-like ones, a clear theoretical picture of the mechanical nature of this behavior is still missing. In this article we address this lack. Using a combination of classical differential geometry, linear stability analysis and computational fluid dynamics, we demonstrate that confined cell layers, here modeled as active polar liquid crystals, are unstable to the formation of protrusions in the presence of a +1 disclination. In extensile systems with positive flow alignment, the instability originates from an interplay between the focusing of elastic forces, mediated by the defect, and a renormalization of the system’s surface tension by the active flow, arising in response to the perpetual injection of active stress under confinement. In the post-transitional phase such competition leads to additional dynamical regimes,
which includes oscillatory deformations, the nucleation of droplets and a turbulent state with proliferating protrusions. By contrast, in contractile systems, the same phenomenon stabilizes the flat configuration, thus preventing the formation of protrusions.

I. THE MODEL

Although a precise account of all the examples of defect-mediate morphogenesis illustrated in the introduction requires, in principle, a great deal of biophysical detail and a case-by-case approach, their very diversity suggests the existence of a general underlying mechanism. Our goal is to identify and rationalize this mechanism in an in-depth analytical description. Our model cell layer consists of a thin film of active polar or nematic liquid crystal, whose mid-surface $M$ is spanned by the unit normal $n$ and the pair of orthogonal tangent vectors $g_i$, with $i = 1, 2$, and $g_{ij} = g_i \cdot g_j$ the associated metric tensor. The mean and Gaussian curvatures are denoted by $\sigma$, $\kappa$ right-hand side of Eq. (2a) correspond, respectively, to the force per unit length $f_{ij}^d$, $f_{ij}^A$, and $f_{ij}^\kappa$. The system free energy is given by

$$F = \int_M \text{d}A \left[ \gamma + \kappa_B H^2 + \kappa_G K + \frac{\kappa_F}{2} \left( \nabla_i p_j \right) \left( \nabla^i p^j \right) \right].$$

The first three terms on the right-hand side of Eq. (1) account for the energetic cost of deformations of the mid-surface, where $\gamma$ is the surface tension, $\kappa_B$ the bending rigidity and $\kappa_G$ the Gaussian-splay modulus $[20]$. The last term, on the other hand, is the Frank free energy $[21]$ expressing the compliance of the system to a local distortion of the cellular polarization, with $\nabla_i$ denoting covariant differentiation and $\kappa_F$ the rotational stiffness in the one-elastic-constant approximation.

The steady state we aim at describing comprises a dense population of cells freely translating and rotating along a stationary mid-surface $M$. The former requirements amount to the following set of hydrodynamic equations for $p$ and the momentum density $\rho v = \rho v^i g_i$ on the tangent plane $[22–25]$

$$\rho (\partial_t + v^k \nabla_k) v^j = \nabla_j \left( \sigma^{ij}_n + \sigma^{ij}_a \right) + f_{ij}^d,$$

(2a)

$$\left( \partial_t + v^k \nabla_k \right) p^i = \left( g^{ij} - p^j p^i \right) \left( \lambda \varepsilon_{ijk} p^k - \omega_{ijk} p^k + \Gamma h_i \right).$$

(2b)

The cellular flow is assumed to be incompressible, thus $\rho = \text{const}$ and $\nabla_i v^i = 0$. The three terms on the right-hand side of Eq. (2a) correspond, respectively, to the hydrodynamic stress tensor $\sigma^{ij}_n = -P_h g^{ij} + 2 \eta u^{ij}$–where $P_h$ is the pressure, $\eta$ the shear viscosity and $u^{ij} = (\nabla^i v^j + \nabla^j v^i)/2$ the strain-rate tensor– the active stress $\sigma^{ij}_a$ and the force per unit length $f_{ij}^d$, originating from the distortion induced by defects and Gaussian curvature, driving the so-called elastic “backflow” $[26]$. The latter can be derived upon expressing the polarization gradients in terms of the geometric potential $\chi$, subject to the Poisson equation $\nabla^2 \chi = K - \rho_d$, where $\rho_d$ is the topological charge density $[25, 27–29]$. Explicitly: $f_{ij}^d = -2 \kappa_F \rho_d \nabla^i \chi$ (see SI). Thus, at equilibrium, $\rho_d \sim K$ so that defects place themselves in regions of like-sign Gaussian curvature in order to minimize the elastic free energy $[29]$. Finally, the active stress tensor embodies the contribution of the force dipoles autonomously generated by the cells and, following a standard construction, can be expressed as $\sigma^{ij}_a = \alpha (p^j p^i - g^{ij} / 2) [3, 30–33]$. The phenomenological constant $\alpha$ quantifies the magnitude of the cellular forces and is positive (negative) for contractile (extensile) systems.

Eq. (2b) governs the rotational dynamics of the cells which, in turn, is dictated by the coupling with the local flow field, expressed by the first two terms on the
right-hand side of the equation, with $\lambda$ the flow alignment parameter and $\omega^{ij} = (\nabla^i v^j - \nabla^j v^i)/2$ the vorticity tensor. The last term is the molecular field $h_1 = -\delta F/\delta \rho = \kappa_p \nabla^2 p_i$ which drives the system towards the ground state of the free energy, with $\Gamma^{-1}$ the rotational viscosity (see e.g. Ref. [21]).

Finally, the stationarity of the mid-surface $\mathcal{M}$ requires the net force acting along the normal direction $n$ to vanish, hence

$$K_{ij} \left( \sigma^{ij}_h + \sigma^{ij}_n \right) + f^a_{\eta} + f^e_{\rho} = 0 , \quad (3)$$

where $K_{ij}$ is the curvature tensor (see Fig. 2a). The normal forces per unit length $f^a_{\eta}$ and $f^e_{\rho}$ on the left-hand side of Eq. (3) are found from minimizing the free energy $F$ (see SI) and are given by $f^a_{\eta} = 2\gamma H + 2\kappa_B H (K - H^2) - \kappa_B \nabla^2 H$ and $f^e_{\rho} = 2\kappa_F (2Hg^{ij} - K^{ij}) \nabla_i \chi \nabla_j \chi + 2\kappa_F (K^{ij} - Hg^{ij}) \nabla_i \chi \nabla_j [25, 34–37]$. We stress that Eq. (3) reduces to the Helfrich shape equation, $f^e_{\rho} = 0$, in the limit $\alpha = \kappa_B = 0$ [19, 34], and to the van Kármán equation, $f^a_{\eta} + 2\gamma H = 0$, in the limit $\alpha = \kappa_B = 0$ [38, 39].

II. MORPHOGENETIC STABILITY DEFECTIVE CELL MONOLAYERS

In this section we investigate how topological defects and activity conspire to render an initially flat cell monolayer unstable to the growth of protrusions. While Eqs. (2) and (3) hold for arbitrary conformations of the mid-surface $\mathcal{M}$, here we consider the simpler case of an axisymmetric surface and a liquid crystal with polar symmetry, where the local polarization and velocity fields depend solely on the distance $r \leq R$ from the symmetry axis, with $R$ the radius of the cell layer: i.e. $p = p(r)$ and $v = v(r)$. Under this assumption the polarization field inevitably features a $+1$ topological defect at the center of the system. Thus, taking $\rho_A = 2\pi \delta(r)$, with $\delta(\cdot)$ a delta-function on $\mathcal{M}$, and setting $\chi = 0$ at $r = R$ without loss of generality, the geometric potential can readily be found to be $\chi = -\log(r/R)$.

To make progress, we parameterize the mid-surface $\mathcal{M}$ in a Monge patch, so that the position $r = r(\varphi)$ of a generic point is given by $r = r g_r + h e_z$, with $e_z$ a unit vector in the $z$-direction and $h = h(r)$ the height. Following a standard approach of membrane physics (see e.g. Ref. [19]) we then assume that $|\nabla h| \ll 1$, so that one can ignore terms of order $O(\nabla^2 h^2)$ and higher in Eqs. (2) and (3). Under such a small-gradient approximation, $\delta_{ij} \approx \delta_{ij}$, $H \approx -\nabla^2 h/2$, $K \approx 0$, and Eqs. (2) reduce to their flat counterpart. Moreover, axial symmetry and incompressibility yield $v^r = 0$, so that $v = 1/r v^\varphi g_\varphi$, with $v^\varphi = v^\varphi(r)$, and $p = \cos \varphi g_\varphi + 1/r \sin \varphi g_\varphi$ with $\epsilon$ a constant and $(r, \varphi)$ polar coordinates on the $h = 0$ plane. Solving Eq. (2b) we find $\epsilon = \pm \arccos(-1/\lambda)/2$, under the assumption that $|\lambda| > 1$. Similarly, neglecting unimportant inertial terms [40] and solving Eq. (2a) for no-slip boundary conditions gives, after standard algebraic manipulations (see e.g. Ref. [41–43]):

$$v^\varphi = \pm \frac{\omega}{\eta} \sqrt{\frac{1 - 1/\lambda^2}{2}} \frac{r \log r}{R} , \quad (4a)$$

$$P_h = -\frac{\omega}{\lambda} \frac{r \log r}{R} - \kappa_F \rho_d , \quad (4b)$$

consistently with a classic result by Kruse et al. [41]. It is worth stressing that, by virtue of Eq. (4b), the pressure nominally diverges at the origin, where the $+1$ defect is located. Like other divergences stemming from the continuous description of defects, however, this one too can be regularized by introducing a core radius $r_c$ setting the short length scale cut-off, so that $r_c \leq r \leq R$. Finally, replacing Eqs. (4) in Eq. (3), gives a stability condition for the flat conformation of the mid-surface $\mathcal{M}$ in the form of a fourth-order linear partial differential equation with position-dependent coefficients:

$$\frac{KB}{2} \nabla^4 h - \gamma_{eff} \nabla^2 h + \frac{\kappa_{eff}}{\alpha} \partial_r \left( \frac{\partial_r h}{r} \right) = 0 , \quad (5)$$

where $\gamma_{eff} = \gamma - P_h$ and $\kappa_{eff} = \kappa_F + \alpha/(2\lambda) r^2$ are effective surface tension and Frank's elastic constant which include the renormalization resulting from the active flow. Note that the velocity field does not enter Eq. (5) explicitly, as the term $K_{ij} v^j$ vanishes identically. Moreover, $\mathcal{M}$ is clamped at the boundary such that $h$ and all its derivatives vanish at $r = R$.

Now, to gain insight into the stability of the system with respect to the formation of protrusions, we separately consider three relevant cases, namely: (i) $\kappa_B = 0$ and $\alpha = 0$; (ii) $\kappa_B = 0$ and $\alpha \neq 0$; (iii) $\kappa_B \neq 0$ and $\alpha = 0$. The first scenario has been considered by Frank and Kardar [44] and corresponds to the case of a passive liquid-crystalline disk-like interface plagued by a $+1$ disclination in the center. In this case Eq. (5) can be integrated exactly, to give

$$(r^2 - R_c^2) \partial_r h = 0 , \quad (6)$$

with $R_c^2 = \kappa_F/\gamma$. Because of our boundary conditions for $h$, this equation admits a nontrivial solution only if $R > R_c = \sqrt{\kappa_F/\gamma}$. In fact, even Eq. (3) is exactly integrable in this case and $\mathcal{M}$ is found to be a parabolic pseudosphere —namely a surface of constant negative Gaussian curvature— whose height and mean curvature diverge as $r \to 0$ [44]. Similarly, in the second scenario, Eq. (5) reduces to Eq. (6), but with $R_c$ given by the solution of the transcendental equation $\kappa_F R_c^2 = \gamma - \alpha/(2\lambda) - \alpha/\lambda \log R/R_c$. As in the previous case, we find that there is a non-trivial solution only if $R > R_c$, thus the flat conformation becomes unstable to the growth of protrusions when the radius $R$ of the cell layer exceeds the critical radius

$$R_c = \sqrt{\frac{\kappa_F}{\gamma - \frac{\alpha}{2\lambda}}} . \quad (7)$$
Eq Note that for $\lambda > 1$ the activity reduces (increases) the effective surface tension in the presence of contractile (extensiile) stresses and vice versa for negative $\lambda$ (see also Ref. [37]). Conversely, if $\gamma < \alpha/(2\lambda)$, Eq. (5) has no non-trivial solutions and the flat solution is always stable. Finally, in the third and most generic scenario, Eq. (5) cannot be reduced to Eq. (6) and one cannot find a stability criterion as simple as that expressed by Eq. (7). Yet, as for the passive instability discussed in Ref. [44], we expect the bending stiffness $\kappa_B$ to merely renormalize Frank’s elastic constant $\kappa_F$. In addition, a finite bending rigidity makes cusps, kinks and other singularities characterized by a diverging curvature energetically prohibitive, hence we expect the tip of the protrusion to become increasingly smooth with increasing $\kappa_B$.

To substantiate the latter statement, we have lifted the small-gradient approximation and numerically integrated Eqs. (2) and (3) on an axisymmetric surface. The equations do not decouple in this limit, therefore it is not possible to condense them in a single equation for the height $h$. However, one finds from the incompressibility equation and with $p = 1/g \cos \epsilon \mathbf{g}_r + 1/r \sin \epsilon \mathbf{g}_\varphi$, where $g = \sqrt{1 + (\partial_r h)^2}$, that $v = 1/r v^r \mathbf{g}_r$, and the angle $\epsilon$ as well as the pressure $P_h$ are left unchanged. The remaining fields, $v^\varphi$ and $h$, can be numerically computed and $h$ is shown in Fig. 2b for different values of activity. It should be noted that the solutions found for $v^\varphi$ are essentially identical to the analytical solutions obtained using the small-gradient expansion.

III. POST-TRANSITIONAL SCENARIO, OSCILLATORY REGIME AND DROPLET NUCLEATION

The interplay between defect-mediated stress focusing and the bending elasticity of the cell layer, illustrated in the previous section, is a striking example of how the entanglement between the topology of the polarization field and the geometry of the flow cooperatively render an initially flat cell layer unstable to the growth of protrusions. In this section, we look at the evolution of the instability to unveil how topological defects influence the morphology of the protrusion in the post-transitional scenario. To this end, we consider a layer of an active polar liquid crystal of thickness $\xi$ confined at the 2D interface between two Newtonian fluids, whose relative concentration is described by the phase field $\phi = \phi(r)$ [45, 46]. In the limit $\xi \to 0$, the simulated interface can be factually interpreted as the mid-surface $M$ of Sec. I [13, 47]. A description of the model is provided in the Materials and Methods section. We numerically integrate the three-dimensional hydrodynamic equations of this diffuse interface model in a cylindrical container with homeotropic boundary conditions along the base, so that the equilibrium configuration is stationary and characterized by a +1 aster at the center of a flat disk. The main control parameters are (i) the dimensionless activity $A = \alpha \ell_c^2 / \kappa_F$, with $\ell_c$ the coherence length of the liquid crystal (see Material and Methods), driving $M$ away from its equilibrium shape and (ii) the reduced surface tension $\Sigma = \gamma \ell_c / \kappa_F$.

As the dimensionless activity $A$ is increased, four different regimes are encountered. At small $A$ values, the surface is flat, whereas the polarization evolves into a spiral configuration coupled to a vortex flow (Fig. 2a), in agreement with our prediction of Sec. II and Ref. [41]. As activity is further increased, the interface undergoes a
Figure 3. Dynamical regimes in active membranes. We use computational fluid dynamics to simulate an active polar interface. We obtain the phase diagram, shown in (f), in terms of dimensionless activity $A$ over reduced surface tension $\Sigma$. We find four different dynamical regimes at varying extensile activity which are illustrated in panels (a-e) with constant surface tension $\Sigma = 0.6$. Each panel is labeled with the respective symbol appearing in the phase diagram. The white vectors are the polarization field, while the color code refers to the local intensity of the flow (color bar in panel (g)). At small activity (yellow pentagon in the phase diagram), we find a vortical flow structure and the interface is flat. This is illustrated in (a) where $A = -0.2$ and the inset shows the spiral pattern in the polarization field. Beyond the threshold of the buckling instability (green squares), the membrane attains a funnel-like profile with the topological defect at the end of the hollow; this is shown in (b). At slightly higher activity, just below the transition to the region of droplet production ($A = -0.4$), we find that the interface begins to oscillates between this funnel-like state and the configuration shown in (c). Next, in panel (d) we show snapshots taken at different times during the process of droplet nucleation at $A = -0.55$ (cyan circles). Finally, in (e), we show a chaotic configuration of the membrane in the active turbulent regime ($A = -1.0$, blue diamonds). Panel (h) shows the time evolution of the normalized distance in the $L_2$ space between the simulated profile of the phase field $\phi$ and the equilibrium one, $\phi^* = \tanh(z/\xi)$, with $\xi$ the equilibrium interface width between the two isotropic fluids and $z$ the coordinate in the direction normal to the interface. The inset on the top-left in panel (h) refers to the case at $A = -0.4$ and $\Sigma = 0.6$, see also Movie S3. The frequency of the oscillation linearly increases with $|\alpha|$ along the transition line, as can be rationalized either by dimensional analysis or from the balance of non-equilibrium stresses and viscous ones. This oscillatory instability lies at the heart of another striking behavior which is observed when activity is further increased. In this case, the surface’s entropic elasticity is no longer able to counteract the active stresses fueling the growth of the protrusion (top panels in Fig. 2d and Movie S4). This results in a steady increase of the passive stresses along the protrusion, which eventually results in the breaking of the interface and the consequent nucleation of a droplet (bottom panels in Fig. 2d and Movie S4). The emulsified droplets are therefore enclosed in a thin active polar shell which separates the buckling instability (light green squares in Fig. 2f), which results in the development of a protrusion, featuring a $+1$ defect at the tip. Thanks to our computational approach, we notice that, once the instability is set by the previously described competition between defect-mediated stress focusing and bending elasticity, the development of non-planar features is further facilitated by the lengthening of the vortical flow field out-of-plane, a process called vortex stretching which occurs in 3D fluids as the result of conservation of angular momentum [48]. The transition line between the flat and buckled configurations in the phase diagram of Fig. 2f shows a linear behavior in the $A - \Sigma$ plane, consistently with the analytical criterion expressed by Eq. (7).

Remarkably, the competition between active and elastic stresses may eventually give rise to large perturbations around the stationary cuspidal profile, with the surface oscillating from the singular configuration with negative curvature of Fig. 2b to the smooth configuration of Fig. 2c. The dynamic of the oscillations can be captured by measuring the temporal evolution of the distance of the simulated interface from the flat configuration, shown in Fig. 2h and its inset for the case at $A = -0.4$ and $\Sigma = 0.6$, see also Movie S3. The frequency of the oscillation linearly increases with $|\alpha|$ along the transition line, as can be rationalized either by dimensional analysis or from the balance of non-equilibrium stresses and viscous ones. This oscillatory instability lies at the heart of another striking behavior which is observed when activity is further increased. In this case, the surface’s entropic elasticity is no longer able to counteract the active stresses fueling the growth of the protrusion (top panels in Fig. 2d and Movie S4). This results in a steady increase of the passive stresses along the protrusion, which eventually results in the breaking of the interface and the consequent nucleation of a droplet (bottom panels in Fig. 2d and Movie S4). The emulsified droplets are therefore enclosed in a thin active polar shell which separates the
interior of the droplet from the outer Newtonian fluid. For each droplet the polarization field develops two boojums (i.e. +1 defects) as required by the Gauss-Bonnet theorem.

Finally, in the limit of very large activity (blue diamonds in Fig. 2f), the dynamics becomes fully chaotic, a regime which is known as active turbulence in the literature [22, 40, 49, 50]. The membrane is characterized by the proliferation of many amorphous protrusions (see Fig. 2e and Movie S5) which elongate under the straining effect of bending deformations in the polarization pattern.

**DISCUSSION AND CONCLUSIONS**

In this paper, we elucidated how the enthralling interplay between topology, geometry and hydrodynamics enables the development of non-planar features in active liquid crystals, as recently observed experiments on biological as well as biomimetic systems and, more prominently, in eukaryotic cell layers (Fig. 1 and Refs. [9–12]). Whereas the amount of biochemical detail necessary for a thorough account of all aspects of this phenomenon often results in a complex tangle which hinders fundamental understanding, here we followed a more generic approach, by focusing on the dynamics at the mesoscopic scale and retaining only the orientational and elastic degrees of freedom, which all realizations of defect-mediated morphogenesis have in common.

By looking at an axisymmetric surface plagued in the center by a +1 disclination, we analytically demonstrated that defective cell layers are unstable to out-of-plane deformations and obtained a stability criterion in terms of system size, orientational stiffness, surface tension and active stresses. This mechanism allows defects to serve as topological morphogens for formation on non-planar features, such as domes and protrusions. Such an instability originates from the competition between the focusing of the elastic forces, mediated by defects, and the renormalization of the system’s surface tension by the active flow. Upon modelling the cell layer as a diffuse active polar interface and turning to computational fluid dynamics, we further investigated the post-transitional regime and constructed an exhaustive phase diagram. At low activity, we recover the results of our analytical approach. Upon increasing the activity, we first find a regime where the membrane oscillates periodically between two different buckled states and then a regime where the high activity breaks up the interface leading to continuous droplet nucleation. Finally, at very large activity, we see a turbulent regime which is characterized by the chaotic proliferation of protrusions.

Whereas undoubtedly simplified with respect to the seeming endless complexity of living matter, our study contributes to shed light on how developing organisms can take advantage of topology to achieve biological organization from physical mechanisms, with potential application to various biomechanical processes such as morphogenesis, embryogenesis, cancerogenesis and vesicle formation. As mentioned in the introduction, +1 disclinations are not the only type of defects encountered in morphogenesis, but nematic (i.e. ±1/2, see Fig. 1a,b) and, in general, p–atic defects (e.g. ±1/6, see Fig. 1d), are equally common and we will investigate them in the future. Finally, while there are several experiments observing the role defects play in the formation of protrusions, we are not aware of any study investigating the other regimes, in particular droplet nucleation. Naturally, the question occurs if these other regimes also play a role in biological systems.

**MATERIALS AND METHODS**

**Experimental setup**

Parental Madin-Darby Canine Kidney (MDCK) GII cells (kindly provided by M. Gloerich, UMC Utrecht) were grown on non-coated coverslips until tissue buckling. F-actin, E-cadherin and nuclei were stained on fixed samples. Samples were imaged at high resolution on a spinning disk confocal microscopy setup. Cell boundaries were identified from a maximum intensity projection of a z-stack of the top part of the dome. Fiji software was used for the orthogonal view. 3D reconstructions were done by ImarisViewer9.7.0 and z-directions were corrected for spherical aberration and axial distortion [51]. See the SI for details.

**Numerical simulations**

The dynamical fields of the model used for simulations are the incompressible flow field \( \mathbf{v} = \mathbf{v}(r,t) \) (\( \nabla \cdot \mathbf{v} = 0 \)) and the polarization field \( \mathbf{P} = \mathbf{P}(r,t) \) which is confined at the interface between two isotropic fluids, whose relative concentration is encoded in the scalar phase field \( \phi = \phi(r,t) \). Note that in this section \( \mathbf{P} \) and \( \mathbf{v} \) are threedimensional vectors and \( \nabla \) now denotes differentiation in \( \mathbb{R}^3 \). The equilibrium is defined by a generalized Landau-de Gennes functional [45, 46]:

\[
\mathcal{F}[\phi, \mathbf{P}] = \int dV \left[ -\frac{a}{2} \phi^2 + \frac{a}{4} \phi^4 + \frac{k_2}{2} (\nabla \phi)^2 + A_0 \left( \frac{\psi}{2} \mathbf{P}^2 + \frac{1}{4} \mathbf{P}^4 \right) + \frac{k_F}{2} (\nabla \mathbf{P})^2 \frac{\beta}{2} (\mathbf{P} \cdot \nabla \phi)^2 \right],
\]

(8)

where the constants \( a \) and \( k_2 \) are model parameters related to the surface tension and the width of the interface by \( \gamma = \sqrt{8ak_2}/9 \) and \( \xi = \sqrt{2k_2/a} \), respectively. To confine the polar liquid crystal at the \( \phi \)-interface the parameter \( \psi = \psi(\nabla \phi) \) is chosen to be a function of \( \nabla \phi \) such that \( \psi = -1 \) if \( |\nabla \phi| \) is larger than a suitable threshold.
and $0$ otherwise. Additionally, the coupling between $P$ and $V\phi$ ensures tangential anchoring of the liquid crystal for $\beta > 0$ so that the polarization field actually lays on the interface. The bulk constant $A_0$ fixes the coherence length of the liquid crystal $\ell_c = \sqrt{\kappa_F/A_0}$ which controls how fast the order parameter $P$ drops to zero from its equilibrium value $|P| = 1$ in proximity of a topological defect. Note that in the limit of vanishing interfacial width, $\xi \rightarrow 0$, such a phase-field model can be mapped on the analytical model of Sec. I (see e.g. Ref. [13, 47]).

In this approach, the dynamical equations governing the evolution of the system are a convection-diffusion equation for the phase field:

$$\partial_t \phi + \nabla \cdot (\phi \mathbf{v}) = \nabla \cdot \left( M \nabla \frac{\delta F}{\delta \phi} \right), \quad (9)$$

where $M$ is the mobility parameter. The hydrodynamic equations for the momentum density $\rho \mathbf{v}$ and the polarization $P$ are the generalization of Eqs. (2) in $\mathbb{R}^3$ and are given in the SI.

The dynamics is solved through a hybrid lattice Boltzmann method integrating the equations in a cylindrical geometry with homeotropic boundary conditions for the polarization field at the boundary [52]. More details on the numerical method, parameters and the preparation of our numerical experiment is provided in the SI together with a mapping between simulation to physical units.

**AUTHORS’ CONTRIBUTIONS**

L.A.H., L.N.C., and L.G. designed and performed research, analyzed data and wrote the paper. L.A.H. and L.G. developed the analytical model, L.N.C. the simulations. J.E. performed MDCK II experiments and analysis.

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