Wrinkling instability in 3D active nematics

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Active matter is a state of matter where large-scale dynamical structures emerge from the interaction of individual active components each driven by their own internal energy source. A well investigated class of such systems comprises active nematics [1, 2] where individual elongated components self-organize into a dynamic state with spatio-temporal chaos with topological defects – a behaviour known as active turbulence. Examples of this phenomenon are given by the dynamics of freely swimming elongated bacteria colonies [3–7], flagella of grafted bacteria or cilia [8], cell monolayers [9, 10], as well as networks of cytoskeletal filaments and motor proteins fueled by ATP [11, 12]. Systems investigated so far have been mostly limited to two-dimensions, and the prospect of developing three-dimensional active matter systems is still largely unexplored. It is therefore interesting to ask how active nematics self-organize in three dimensions. Here we show experimentally that a three-dimensional suspension of microtubules and kinesin-1 motors self-organizes into an active nematic ribbon that for a range of parameters wrinkles periodically before transitioning to a state of active turbulence. We understand the observed behaviour with stochastic simulations and a quantitative theory that describes the wrinkling instability. Our results show that 3D active nematics promise insights into novel 3D active matter.

RESULTS AND DISCUSSION

We experimentally investigate the dynamics of an active nematic, confined in a long rectangular channel of size 30 mm × 1.5 mm × 100 μm. The active nematic consists of an ionic aqueous solution of microtubules, multi-headed kinesin-1 motors, and poly(ethylene glycol) (PEG) as depletion agent [12]. Internally generated spatio-temporally chaotic flows at the millimetre scale were observed that persisted as long as ATP was available. The networks exhibit a steady state with permanent flow at large scales and the dynamics at the microscopic scale is driven by the buckling and elongation of the microtubule bundles due to the activity of the motors [13]. At high concentrations these networks show a transition to a locally ordered nematic liquid-crystalline state with topological defects and spatio-temporal dynamics [14,15] that can be varied by tuning experimental parameters [21]. In the past 3D confinement in spherical vesicles, toroids, disks, and other complex geometries were employed and it was observed that the generation of coherent flows could provide dynamic motility on the surface of the vesicles or produce a laminar flow within the given geometries. Our work shows another feature of active nematics, namely the evolution of a 3D space-filling system to a contracted ribbon that wrinkles in the third-dimension due to its longitudinal extension before making a transition to active turbulence.
FIG. 1. 3D wrinkling formation. (a) Schematic representation of the wrinkling process as a result of contractile and extensile forces that emerge at different times: the 3D active nematic system evolves to exhibit contraction into a sheet, a wrinkling instability simultaneously as a lateral contraction, and a final transition to active turbulence with spatio-temporal chaos. The red arrow indicates the nematic director before the onset of wrinkling instability. The black arrows indicate the elastic deformations induced by the active stress along different directions. (b-e) time evolution of 3D active nematics through the different stages. The nematic order along the x-direction is visible. Scale bar: 500 µm. (f-g) Pattern visualization at two different heights. The 3D structure can be seen by the wave crests in focus and the adjacent ones out-of-focus when the focal plane is set to z = 0 µm. At z = 70 µm the situation is reversed. Scale bar: 250 µm.

so far. Inside the ribbon nematically ordered filament bundles are clearly visible. The first mode of evolution of the active nematic is contraction perpendicular to the nematic director. This leads to a buildup of the extensile stress along the nematic director \( \mathbf{\Pi} \) (Fig. 1a), which can then lead to a spatially periodic wrinkling of the ribbon (Fig. 1b-e). We find that the development of the extensile stress that is needed for this phenomenon to occur requires the combined action of the multiheaded kinesins and the depletion forces (see Methods section). The amplitude of the wrinkling grows with time. After the time \( \tau_{at} \), the system reaches a dynamic state of active turbulence. The characteristics of the material needed to form a 3D active nematic differ significantly from those in the previously well studied 2D situations [12]. Whereas those studies typically use short microtubules with lengths around 1 µm, nematic ordering and active stress buildup in 3D systems, which have a relatively low volume fraction of microtubules (0.001), require longer filaments. The average microtubule length in our experiment was 19 µm ± 10 µm. We verified the importance of length by shearing the filaments to much shorter lengths, in which case no pattern was observed; instead we observed an irregular mesh with regions of increased local aggregation. In our experiments at onset of the wrinkling instability the observed wavelength \( \lambda \) ranged from 200 µm to 500 µm and the time until the onset of instability (\( \tau_w \)) varied between 10 minutes and 2 hours (Fig. 21).

We find that the wavelength of the pattern and the
time scale of its formation are correlated – wrinkling instabilities that appear at shorter times tend to have shorter wavelengths than those forming later. To further investigate this correlation, we varied the ATP concentration from 2 mM, at which the motors will be reaction-limited, to 10 μM that is in the diffusion-limited regime. Contrary to what one might expect, no discernible effect on the wavelength of the wrinkling instability could be observed. At 1 mM ATP concentration the wavelength of the pattern was 272 μm ± 36 μm and the wrinkling set in after few minutes; at 10 μM ATP the wavelength was 249 μm ± 38 μm and the onset of wrinkling occurred at around 13 minutes. It is clear that the variation of the characteristic wrinkling formation times cannot be understood through the biochemical process linking velocity and force to ATP hydrolysis, as is the case in classical 2D active nematics [23]. The times and wavelengths follow the same trend that was observed when considering the variability of outcomes under the same ATP concentration. These observations suggest that the active stress is determined by the stall force of kinesin motors that is independent of the ATP concentration as well as the cross-linker nature of the motors. To test the importance of the motor concentration, we decreased the kinesin concentration by 10-fold (to 1.7 nM) and indeed did not observe the instability (Fig. 2e). The network contracted continuously for around 48 hours resembling the contraction of the stabilized microtubule network in *Xenopus* oocyte extracts [24] and the resulting deformation along the y-axis was comparable to the case with
higher motor concentration. Although the active stress in this is not sufficient to induce the wrinkling of the network, significant active force is exerted on the system via cross-linking of the motors as long as ATP is available. We can develop a theoretical understanding of the observed phenomena based on the combination of the contraction and the mechanical activity inside the resulting ribbon. The director field is mostly aligned in the direction of the channel, \( \hat{n} \sim \hat{e}_x \). For microtubules and kinesin motors, we expect an extensile stress along the director field (\( \zeta > 0 \)). This stress is accompanied by contractile stresses across the channel (directions \( \hat{e}_y \) and \( \hat{e}_z \)). After the contractile stress in the \( z \)-direction leads to the formation of the ribbon, we can describe the system as a two dimensional active nematic sheet that can explore out-of-plane bending deformations. For this effective description, the nematic order is described by the symmetric traceless tensor \( \mathbb{Q} = (\hat{n} \otimes \hat{n} - \frac{1}{2} \mathbb{I}) \mathbb{S} \), with the order parameter \( \mathbb{S} \), which has the value 1 for perfect nematic alignment and 0 for an isotropic state. The active stress that is caused by the motors can be written as \( [2] [25] \)

\[
\sigma_{\text{active}} = -\zeta \mathbb{Q} .
\]  

The ribbon can be described by a quasi 2D sheet, whose shape can be described (in a Monge representation) by a function \( h(x, y) \), and a sheet stiffness \( \kappa_{\text{eff}} \) that can be derived from the bending modulus of the individual filaments and the nematic order parameter (see Appendix B). Focusing for simplicity on the variations along the \( x \)-axis, we can construct a generalized free energy functional as

\[
\mathcal{F}[h] = \frac{1}{2} \int dA \left[ \kappa_{\text{eff}} (\partial_x^2 h)^2 + \sigma_{x}^{\text{active}} (\partial_x h)^2 \right] \]  

where the coupling between the active stress and the deformation arises from the nonlinear contribution to the strain tensor (see Appendix B for a full derivation that includes deformation in both directions). The emerging instability of the sheet is subject to viscous dissipation (either in the fluid, or in the remaining microtubule network outside of the sheet). We describe it with a local drag term that resists vertical movement of the sheet through water, which is written as \( F \partial_x h \). Within a linear stability analysis, we use the ansatz \( h(x, t) \propto e^{i\mathbf{q} \cdot \mathbf{x}} \) for a perturbation with the wave vector \( \mathbf{q} \). We find a growth rate of

\[
\mathcal{A}(q) = \frac{1}{\Gamma} \left( -\kappa_{\text{eff}} q^4 + \frac{1}{2} \zeta_S q^2 \right) .
\]  

For negative values of the active stress corresponding to extensile stress, the growth rate \( \mathcal{A}(q) \) will be positive for an interval of wave numbers, with the fastest growing mode having the wave number \( q^* = \sqrt{\frac{\zeta_S}{4\kappa_{\text{eff}}}} \) and a growth rate

\[
\mathcal{A}(q^*) = \frac{\zeta^2 S^2}{8\Gamma \kappa_{\text{eff}}} .
\]  

Assuming that the samples do not differ in other parameters, we obtain a relationship between the characteristic time of pattern formation and the wavelength \( \lambda = 2\pi/q^* \) as follows

\[
\tau_w \propto \lambda^4
\]  

The experimental data shown in Fig. 2d confirm the trend predicted by Eq. (4), both when comparing the outcome in equal samples and in those with different ATP concentrations. We also note that the dependence of the active stress on the local nematic order is manifestly demonstrated in samples with spatial non-uniformity, where wrinkling is always in the direction of the nematic director and regions with wrinkling following different orientations can coexist (see Fig. 2f for an example).

We used a complementary computer simulation to directly verify that sheet formation and wrinkling arise from the interplay between the action of kinesin motors and attractive depletion forces. We simulated the dynamics of 20,000 filaments in a sufficiently large box to reproduce the salient phenomena (see Methods). The simulated system consists of elastic filaments (microtubules), tetrameric kinesin motors (pairs of kinesin dimers that can bind to two adjacent filaments) and passive cross-linkers. Filaments are subject to hard-core repulsion and attractive depletion forces. The simulations use a Langevin-dynamics algorithm from the Cytosim package (see Methods). The results are shown in Figure 3.

Starting from a nematic ordered state (Fig. 3a), the simulation first shows the condensation in the middle of the channel and shrinking in the lateral direction (Fig. 3b). As in the experiment, formation of microtubule bundles also becomes visible at this stage. Afterwards, the extensile stress leads to a wrinkling instability in the sheet (Fig. 3c). The growth of the selected out-of-plane deformation mode continues until the amplitude becomes limited by the simulation box size (Fig. 3d). The simulation shows a very good qualitative agreement with the experimental observations. We present a 3D active system without substrate interactions that collapses into a sheet due to activity and further evolves to exhibit a 3D wrinkling instability before transitioning to fully developed active turbulence with defect proliferation. It is interesting to note that no hydrodynamic interactions are necessary for the theoretical description of the emergence of the instability. The active stress is determined by the stall force of the molecular motors and simultaneous cross-linking function as the simulation confirmed. We expect our observation and explanation of these novel emergent properties to broaden the perspective of active nematic systems in 3D.
Initially, the 3D volume is filled with nematically ordered filaments (with orientations within $\theta_{\text{max}} = 30^{\circ}$ of the x-axis), mixed with tetrameric active motors and passive cross-linkers. Filaments are subject to repulsive hard-core interactions and attractive depletion forces. b) The filaments first form a ribbon that contracts laterally. c) Due to extensile stress, the ribbon forms wrinkles. At the same time, increased bundling of filaments is visible. d) The wrinkles grow until they get constrained by the walls of the channel. Upper row: top view; lower row: side view.

**MATERIALS AND METHODS**

**Motile Bundle Solution** The motile bundle solution was prepared as described before [26]. Briefly, the kinesin-streptavidin complexes were prepared by mixing 0.2 mg/ml kinesin 401, 0.9 mM dithiothreitol (DTT), 0.1 mg/ml streptavidin (Invitrogen, S-888) dissolved in M2B. The microtubule mixture was prepared by mixing 2.7 mg/ml 647 HiLyte™ labeled porcine brain tubulin (Cytoskeleton, Inc., U.S.A.) in M2B with 5 mM MgCl$_2$, 0.1 mg/ml streptavidin (Invitrogen, S-888) dissolved in M2B. The microtubule mixture was prepared by mixing 2.7 mg/ml 647 HiLyte™ labeled porcine brain tubulin (Cytoskeleton, Inc., U.S.A.) in M2B with 5 mM MgCl$_2$, 0.1 mg/ml streptavidin (Invitrogen, S-888) dissolved in M2B, and 1 mM GTP, 50% DMSO and 0.3% PEG. The final mixture consists of microtubules and motor proteins and a stabilising mixture composed of 68 mM MgCl$_2$ and 5 $\mu$M taxol. This solution was kept in the oven for 30 minutes at 37° C and introduced into the PDMS channel thereafter. A more detailed description is available in the Supplementary methods. Unlike previous experiments with 2D active nematics [12] that used short microtubules polymerized with GMPCPP (Guanosine-5'-[(\alpha,\beta)-methyleno]triphosphate, a GTP analogue), we used longer microtubules with an average length of 19 $\mu$m ± 10 $\mu$m, polymerized with GTP and stabilized with taxol (see Supplementary methods). By adding the non-adsorbing polymer PEG, attractive interactions between microtubules are induced through depletion force and lead to bundle formation [27] [28]. In our case the bundles had an average length around one order of magnitude longer than the single filaments they are assembled from. The motors are biotinylated and by using streptavidin we assembled them in multihedled clusters up to tetrameric configuration [29] [30]. After the polymerization of microtubules, the active solution was injected into a PDMS microfluidic channel with a rectangular cross-section. The injection was accomplished at low pressure by using a 50 $\mu$l syringe in order to avoid shear damage to the filaments. In order to reduce unspecific protein adsorption the channel was functionalized with PLL-g-PEG (see Supplementary methods for detailed description). By acquiring consecutive areas along the x-axis of the confinement we showed that the 3D instabilities are not local but rather emerging in the whole network. The bundling of microtubules during the polymerization is a requirement for the pattern formation. Indeed, when carrying out the experiment with a microtubule network polymerized without the addition of PEG, no pattern was observed. However, we could observe that the active system results in a compact structure such a gel without any visible network mesh. The peak-to-peak amplitude of the wrinkling can be determined by vertical scans as the difference between the two z-positions at which the structure is in focus (Fig. 1). In the lower plane, half of the 3D wave is in focus and the second half is out of focus. The situation is reversed in the upper plane, demonstrating that at this stage the wrinkles have almost filled the available space.

**Simulation** We simulated the evolution of a 3D active nematic system using the open source Cytosim package [31] (www.cytosim.org). In the simulation we kept a filament density close to the experimental value, but chose a smaller box which was sufficiently large to capture the wrinkles while keeping the demand for memory and CPU time feasible. We thus simulated a box of 200 $\mu$m length, 100 $\mu$m width and 20 $\mu$m height with periodic boundary conditions in x direction and repulsive boundaries in y and z directions. The box contained 20,000 microtubules and the same number of tetrameric kinesins motors and passive cross-linkers. Based on the experiment, the filament length was chosen as 15 $\mu$m. The filaments are subject to repulsive forces when the distance between their centerlines is closer than $d_0 = 50$ nm. Beyond that distance, there is an attractive interaction up to a distance $d_0 + r_{\text{att}} = 90$ nm, which describes the depletion forces caused by the PEG solution. Kinesin motors attach to one or two microtubules with the rate $r_{\text{on}} = 5 s^{-1}$ when they are within a range of 100 nm. When bound, they move along each filament with a prescribed linear force-velocity relationship. The detachment from microtubules is stochastic with a constant unbinding rate $r_{\text{off}} = 0.1 s^{-1}$. Passive cross-linkers behave...
in a similar way as the motors, but do not move along microtubules. The simulation ran in steps of $\Delta t = 0.02$ s until the formation of wrinkles.

APPENDIX A

Supplementary methods

**Microfluidic device.** The experimental chamber was constituted by a custom-made micro-fluidic device made of poly-dimethyl-siloxane (PDMS, 10:1 mixture with curing agent, Sylgard 184, Dow Corning Europe SA). Standard soft lithography was used to produce the micro-fluidic channel that was 1.5 mm wide, 100 $\mu$m high, and 30 mm long. Inlets and outlets for the active mixture were punched through the PDMS by using a syringe tip.

**Non-adsorbing surface coatings and experimental chamber assembly.** Glass coverslips ($64 \times 22 \text{mm}^2$, VWR) were cleaned by sonication in a 2 % Hellmanex III solution (Hellma Analytics) for 30 minutes. Afterwards, they were extensive washed in deionized water, incubated 10 minutes in acetone, 10 minutes in ethanol, extensive washed in deionized water and drying with a filtered airflow. The cleaned coverslips were immediately activated in oxygen plasma (FEMTO, Diener Electronics, Germany) for 30 s at 0.5 mbar and sealed to the PDMS, 0.1 mg/mL Poly-L-lysine)-(graft-poly(ethylene glycol) (PLL-g-PEG) (SuSoS AG, Switzerland) in 10 mM HEPES, pH 7.4, at room temperature was injected into the channel and incubated for 1 h. Finally, M2B buffer was used to remove the excess PLL-g-PEG from the channel.

**Motile bundle solution.** The motile bundle solution was prepared as described before \[26\]. Kinesin 401 was purified as previously published \[32, 33\] and the kinesin-streptavidin complexes were prepared by mixing 0.2 mg/ml kinesin 401, 0.9 mM dithiothreitol (DTT), 0.1 mg/ml streptavidin (Invitrogen, S-888) dissolved in M2B (80 mM PIPES, adjusted to pH = 6.9 with KOH, 1 mM EGTA, 2 mM MgCl$_2$) and incubated on ice for 15 min. The plasmid that codes biotin-labeled kinesin 401 (Kinesin 401-BIO-6xHis) was a gift from Jeff Gelles (pWC2 - Addgene plasmid # 15960; http://n2t.net/addgene:15960; RRID:Addgene_15960) \[33\] and was purified at the Dortmund protein facility (DPF). The active mixture (AM) was obtained by mixing 2.4 mM Trolox (Sigma 238813), 1.7 $\mu$l pyruvate kinase/lactic dehydrogenase (PK/LDH, Sigma, P-0294), 32 mM phosphoenol pyruvate (PEP, VWR AAB20358-06), 16.6 $\mu$l 3% PEG, 5.5$l$ M2B and 3.25$l$ M2B DTT (10 mM), 0.5 mg/ml glucose, 0.2 mg/ml glucose oxidase (Sigma G2133), 0.05 mg/ml catalase (Sigma C40), 2 mM ATP and 4$l$ kinesin1-streptavidin clusters. The microtubule mixture (MT) was prepared by mixing 2.7 mg/ml 647 HIlYte$^\text{TM}$ labeled porcine brain tubulin (Cytoskeleton, Inc., U.S.A.) in M2B with 5 mM MgCl$_2$, 1 mM GTP, 50 % DMSO and 0.3% PEG. The final mixture consists of 15$l$ MT, 29$l$ AM and 5.8$l$ of a stabilising mixture composed by 68 mM MgCl$_2$ and 5 $\mu$M taxol. This solution is kept in the oven for 30 minutes by 37°C and introduced into the PDMS channel thereafter.

**Imaging and tracking.** Image acquisition was performed using an inverted fluorescence microscope Olympus IX-71 with a 4× objective (Olympus, Japan) and a DeltaVision imaging system (GE Healthcare). For excitation, a Lumen 200 metal arc lamp (Prior Scientific Instruments, U.S.A.) was applied. The data was recorded with a CCD camera (CoolSnap HQ2, Photometrics). The images were acquired with a variable frame rate according to the experiment with an exposure time of 500 ms for a variable time according to the experiment. The wavelengths were measured by using ImageJ. In each experiment the wavelength was determined along 5 longitudinal lines at different y positions within the sample. The wavelength values are plotted as mean ± SD for each sample.

APPENDIX B

**Stability analysis of the active elastic sheet**

In the following we present a more general discussion of the dynamics of an active nematic sheet. The calculation takes into account the active stress, anisotropic in-plane elasticity and anisotropic bending stiffness of the sheet. We consider an elastic sheet that spans the $x-y$ plane before deformation. We describe in-plane deformations with the functions $u_1(x,y)$ and $u_2(x,y)$ and the out-of-plane deformation with the function $h(x,y)$. To the leading order, the strain in the deformed sheet is given by

$$u_{ij} = \frac{1}{2} [\partial_i u_j + \partial_j u_i + (\partial_i h)(\partial_j h)]$$

and consists of a linear term for in-plane deformations $u_{ij}' = \frac{1}{2} (\partial_i u_j + \partial_j u_i)$ and a quadratic term $\frac{1}{2} (\partial_i h)(\partial_j h)$ describing the strain caused by small out-of-plane deformations. The curvature tensor is defined by

$$H_{ij} = \frac{\partial_i \partial_j h}{\sqrt{1 + (\nabla h)^2}} \approx \partial_i \partial_j h$$

and determines the mean curvature $\frac{1}{2} h_{ii} = \frac{1}{2} \Delta h$ and the Gaussian curvature $K = \text{det} H$. Note the use of summation convention over identical indices.

In the case of an isotropic sheet, the elastic energy of in-plane deformations is

$$F_{el} = \int dA \left[ \frac{\lambda}{2} u_{ii} u_{jj} + \mu u_{ij} u_{ij} \right]$$

where $\lambda$ and $\mu$ are Lamé’s first and second parameter, respectively.
We describe the nematic order with the symmetric tensor $Q = (\hat{n} \otimes \hat{n} - \frac{1}{3}I)S$, with $\hat{n}$ denoting the director. $S$ is the order parameter, which is 1 if all filaments are aligned in the direction $\hat{n}$. To describe the material elasticity, we introduce the 4-th order anisotropic stiffness tensor $C_{ijkl}$. By definition, $C$ has the symmetries $C_{ijkl} = C_{klji} = C_{jkil} = C_{ijlk}$. It also has to share the symmetries of the $Q$-tensor. We therefore use the following ansatz:

$$C_{ijkl} = \chi Q_{ij}Q_{kl} + \gamma(Q_{ij}\delta_{kl} + \delta_{ij}Q_{kl}) + \lambda \delta_{ij}\delta_{kl} + \mu(\delta_{ik}\delta_{jl} + \delta_{il}\delta_{jk})$$

(9)

The anisotropic elastic energy follows as

$$\mathcal{F}_{cl} = \frac{1}{2} \int dA \ C_{ijkl} u_{ij} u_{kl}.$$  
(10)

In an analogous way, we write the bending energy of the isotropic sheet as

$$\mathcal{F}_b = \int dA \left[ \frac{\kappa}{2} H_{ii} H_{jj} + \bar{\kappa} K \right]$$  
(11)

and generalize it to the anisotropic case as

$$\mathcal{F}_b = \frac{1}{2} \int dA \ \kappa_{ijkl} H_{ij} H_{kl}$$ 
(12)

with

$$\kappa_{ijkl} = \alpha Q_{ij}Q_{kl} + \nu (Q_{ij}\delta_{kl} + \delta_{ij}Q_{kl})$$

$$+ (\kappa + \bar{\kappa}) \delta_{ij}\delta_{kl} - \frac{\bar{\kappa}}{2} (\delta_{ik}\delta_{jl} + \delta_{il}\delta_{jk}).$$

(13)

The active stress also follows the symmetry of the $Q$-tensor and we write it as

$$\sigma_{ij}^{\text{active}} = -\zeta Q_{ij}.$$  
(14)

A positive value of $\zeta$ implies that the stress is extensible in the nematic direction; for a negative $\zeta$ it would be contractile. It is also possible that the activity induces an additional isotropic stress (e.g., make the sheet contract in all directions), but we skip it here as it will not change the qualitative features of the final result. The active stress adds a contribution

$$\mathcal{F}_{\text{active}} = -\int dA \ \sigma_{ij} u_{ij}$$

$$= -\int dA \left[ \zeta Q_{ij} u_{ij}^{t} + \frac{\zeta}{2} Q_{ij}(\partial_i h)(\partial_j h) \right]$$  
(15)

to the total generalized free energy. We can again split the generalized free energy into a part that depends on the in-plane deformations and a part that depends on the out-of-plane deformations:

$$\mathcal{F}_{cl}^{\text{active}} = \int dA \left[ \frac{1}{2} C_{ijkl} u_{ij} u_{kl} - \zeta Q_{ij} u_{ij}^{t} \right]$$

(16)

$$\mathcal{F}_b^{\text{active}} = \frac{1}{2} \int dA \ [\kappa_{ijkl} H_{ij} H_{kl} - \zeta Q_{ij}(\partial_i h)(\partial_j h)].$$  
(17)

In the absence of external constraints or other forces, the elastic part is minimal when the sheet assumes a stretched configuration, which is determined by the equation

$$C_{ijkl} u_{kl}^{\text{eff}} - \zeta Q_{ij} = 0,$$  
(18)

which represents a system of linear equations. In the simplest case of an isotropic elasticity ($\chi = \gamma = 0$), the solution is

$$u_{ij}^{\text{eff}} = \frac{\zeta}{2\mu} Q_{ij},$$  
(19)

i.e., extension along the nematic direction and contraction orthogonal to it.

We expand the bending energy in the Fourier space

$$\mathcal{F}_b^{\text{active}} = \frac{1}{2} \int \frac{d^2 \mathbf{q}}{(2\pi)^2} \left[ \alpha (Q_{ij} q_i q_j)^2 + \nu Q_{ij} q_i q_j \mathbf{q}^2 + \kappa \mathbf{q}^4 - \zeta Q_{ij} q_i q_j \right] |h(\mathbf{q})|^2$$  
(20)

and assume that the sheet is subject to local viscous drag, such that the dissipation rate can be written as

$$D = \frac{1}{2} \int dA \ \Gamma |\partial_i h|^2 = \frac{1}{2} \int \frac{d^2 \mathbf{q}}{(2\pi)^2} \ \Gamma |\partial_i h| |\mathbf{q}^2|^2.$$  
(21)

Then the dynamics of the mode with the wave vector $\mathbf{q}$ is determined by

$$\partial_t h(\mathbf{q}, t) = -\frac{1}{\Gamma} \left[ \alpha (Q_{ij} q_i q_j)^2 + \nu Q_{ij} q_i q_j \mathbf{q}^2 + \kappa \mathbf{q}^4 - \zeta Q_{ij} q_i q_j \right] h(\mathbf{q}, t)$$  
(22)

with the growth rate

$$\Lambda(\mathbf{q}) = -\frac{1}{\Gamma} \left[ \alpha (Q_{ij} q_i q_j)^2 + \nu Q_{ij} q_i q_j \mathbf{q}^2 + \kappa \mathbf{q}^4 - \zeta Q_{ij} q_i q_j \right] .$$  
(23)

With the nematic order along $x$-axis ($\hat{n} = \hat{e}_x$), we have $Q = \frac{2}{\pi} \begin{pmatrix} 1 & 0 & 0 & -1 \end{pmatrix}$ and

$$\Lambda(\mathbf{q}) = -\frac{1}{\Gamma} \left[ \frac{\alpha S^2}{4} (q_x^2 - q_y^2)^2 + \frac{\nu S}{2} (q_x^4 - q_y^4) + \kappa (q_x^2 + q_y^2)^2 - \frac{\zeta S}{2} (q_x^2 - q_y^2)^2 \right].$$  
(24)

Because the last term is quadratic in $\mathbf{q}$, whereas all others are 4-th power, it will dominate at small $\mathbf{q}$ and it is always possible to find wave vectors with a positive growth rate, i.e., unstable modes. The growth rate is always maximal in $x$-direction. On that axis, it reads

$$\Lambda(q_x) = -\frac{1}{\Gamma} \left[ \frac{\alpha S^2}{4} + \frac{\nu S}{2} + \kappa \right] q_x^4 - \frac{\zeta S}{2} q_x^2.$$  
(25)

With $\kappa_{\text{eff}} = \kappa + \frac{1}{4} \nu S + \frac{1}{4} \alpha S^2$, we obtain the simplified equation derived in the main text.
**Acknowledgments**  E.B., I.G., R.G. acknowledge support from the MaxSynBio Consortium which is jointly funded by the Federal Ministry of Education and Research of Germany and the Max Planck Society. E.B. acknowledges support from the Volkswagen Stiftung (priority call “Life?”). A.V. acknowledges support from the Slovenian Research Agency (grant no. P1-0099).

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