Growth-induced self-organization in bacterial colonies

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Chapter 2

Models

2.1 Discrete model

A growing bacterial colony is a typical complex system. Even in a simple setup (e.g. Fig. 1.5), there could be plenty of biochemical and mechanical interactions involved. Depending on the local arrangement of cells, mechanical interactions between neighboring cells may include steric repulsion if squeezing each other, cell-cell adhesion resulting especially from the molecular complexes known as *adhesins*, and frictional forces due to the relative motion [56, 65]. Similar types of forces can be found between cells and substrate [56, 65]. All these mechanical interactions originate from elastic contacts between soft bodies, i.e. cells and substrates, making it even more difficult to determine the magnitude and direction of the forces. In addition, cell growth, as the ultimate driving force, not only depends on the metabolic state of each cell, but also the local concentration of nutrient, which has its own spatial-temporal pattern controlled by the diffusive dynamics [62]. Modeling the system in its full complexity, would be far beyond the capabilities of simple models with few control parameters.

Here, we use a minimal model including only the ingredients that are essential to the dynamics of the system. Each bacterium is modeled as a spherocylinder with a fixed diameter d_0 and a time-dependent length l (excluding the caps on both ends, Fig. 2.1) [62]. The model is in general three-dimensional, but one can enforce it to be quasi-1D or quasi-2D by suppressing specific degrees of freedom. Each cell has a position $\boldsymbol{r}_i$ (the center of mass) and an orientation $\boldsymbol{p}_i$, which is a unit vector pointing from the cell center to either end of the cell. Although the two ends of the cell might have different biochemical or mechanical properties [65, 70], here in this thesis, we assume the cell to be symmetric, and hence $\boldsymbol{p}_i = -\boldsymbol{p}_i$.

Cell growth and division are modeled as following. The length l_i in-

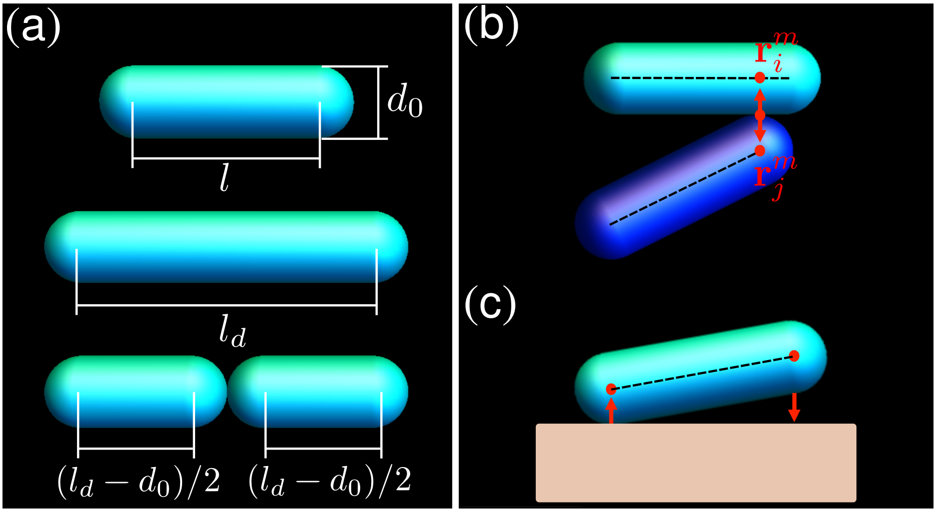

Figure 2.1. Schematics of the hard-rod model. (a) Each cell has a fixed diameter d_0 and a time-dependent length l (excluding the two caps) that increases linearly in time as demanded in Eq. 2.1. Once they reach the division length l_d, they divide into two identical daughter cells. (b) The steric interaction between neighboring cells is modeled as the Hertzian repulsion between two spheres of diameter d_0, centered respectively at $\boldsymbol{r}_i^m$ and $\boldsymbol{r}_i^m$, which minimize the distance between the cell axes (i.e. the two black dashed lines). (c) Each cell interact with the substrate through their caps. The forces on the two caps are calculated independently. They could be repulsive in case of penetration (left end), or attractive in presence of gap (right end).

creases linearly in time,

$$\frac{\mathrm{d}l_i}{\mathrm{d}t} = g_i, \tag{2.1}$$

where g_i is the growth rate of the ith cell. After it reaches the division length l_d, the cell divides into two identical daughter cells. In order to avoid synchronization of divisions, the growth rate of each cell is randomly drawn from a uniform distribution in the interval $[g/2, 3g/2]$, hence g is the average growth rate. Immediately after duplication, the daughter cells have the same orientation as the mother cell but independent growth rates. The rate of cell division can vary over time, with the increase of growth-induced local pressure [71, 72]. In bacterial colonies, however, such an effect takes place only at pressure values that are significantly larger than those experienced by the cells in a microcolony [73, 56] and has, therefore, been neglected in our model.

Neighboring cells, when overlap, interact stericly through a Hertzian-like contact force. To determine the direction and magnitude of the repulsion force, we first find the two points on the major axes of the two cells (black dashed lines in Fig. 2.1b), $\boldsymbol{r}_i^m$ and $\boldsymbol{r}_j^m$, which minimize the distance between the two major axes, hence maximize the overlap of the two cells. The force between the two rods is approximated as a force between two spheres of diameter d_0, centered at $\boldsymbol{r}_i^m$ and $\boldsymbol{r}_j^m$, respectively [62]. Specifically, the force from the jth cell to the ith is $\boldsymbol{F}_{ij}^c = Y_c d_0^{1/2} h_{ij}^{3/2} \boldsymbol{N}_{ij}$, where Y_c is proportional to the Young's modulus of the cell, $h_{ij} = d_0 - |\boldsymbol{r}_i^m - \boldsymbol{r}_j^m|$ is the overlap distance between the two cells, and $\boldsymbol{N}_{ij} = (\boldsymbol{r}_i^m - \boldsymbol{r}_j^m)/|\boldsymbol{r}_i^m - \boldsymbol{r}_j^m|$ the unit vector from $\boldsymbol{r}_j^m$ to $\boldsymbol{r}_i^m$. The point of contact is assumed to be at $\boldsymbol{r}_{ij} = (\boldsymbol{r}_i^m + \boldsymbol{r}_j^m)/2$.

Mechanical forces from the substrates, including the glass slide and the agarose gel on top, can be modeled implicitly, as if they were exerted from an imaginary plane spanning in the x and y directions, at $z = 0$. From now on, we refer to this imaginary plane as the "substrate" for convenience. Cells interact with the substrate through their caps, at positions $\boldsymbol{r}_{i\alpha} = \alpha l_i \boldsymbol{p}_i/2$ ($\alpha = \pm 1$) with respect to the cell center $\boldsymbol{r}_i$, and the force on each cap from the substrate is calculated independently (Fig. 2.1c). This force can be either repulsive or attractive, depending on the positions of the cap centroids, in such a way to model the impenetrability of the glass slide as well as the vertical repulsive force from the agarose gel. If $z_{i\alpha} < d_0/2$, where $z_{i\alpha}$ is the $z-$coordinate of the caps, the cell cap overlaps the substrate, hence is repelled with a Hertzian force $\boldsymbol{F}_{i\alpha}^s = Y_s d_0^{1/2}(d_0/2 - z_{i\alpha})^{3/2}\hat{\boldsymbol{z}}$, where Y_s is an effective elastic constant depending on the Young's modulus of the cell and the substrate. If on the other hand, there's a gap between the cell cap and the substrate, i.e. $d_0/2 < z_{i\alpha} < d_0/2 + r_a$, a vertical restoring force $\boldsymbol{F}_{i\alpha}^s = k_a l_i (d_0/2 - z_{i\alpha})\hat{\boldsymbol{z}}$ will be applied to the cell cap, with r_a the range of the restoring force (Fig. 2.1c). Here, the vertical restoring force can represent either the compression force from the agarose gel on top, or the adhesive forces from the glass/ECM, or a combination of both [56, 65, 66]. In presence of rigid wall confinement in the lateral direction, the repulsive force (no attractive force in this case) from the rigid wall can be calculated in the same way, but the magnitude of the force is proportional to Y_c instead of Y_s.

Since the system is highly overdamped, inertia plays a minor role here. Hence, the motion of cells is governed by the over-damped Newton equations for a rigid body [74], namely,

$$\frac{\mathrm{d}\boldsymbol{r}_i}{\mathrm{d}t} = \frac{1}{\zeta l_i}\left(\sum_{j=1}^{N_i^c}\boldsymbol{F}_{ij}^c + \sum_{\alpha=\pm1}\boldsymbol{F}_{i\alpha}^s + \boldsymbol{\eta}_i\right), \tag{2.2a}$$

$$\frac{\mathrm{d}\boldsymbol{p}_i}{\mathrm{d}t} = \frac{12}{\zeta l_i^3}\boldsymbol{M}_i \times \boldsymbol{p}_i \tag{2.2b}$$

$$\boldsymbol{M}_i = \sum_{j=1}^{N_i^c}(\boldsymbol{r}_{ij} \times \boldsymbol{F}_{ij}^c) + \sum_{\alpha=\pm1}(\boldsymbol{r}_{i\alpha} \times \boldsymbol{F}_{i\alpha}^s). \tag{2.2c}$$

The first term on the right-hand side of Eq. 2.2a represents the repulsive forces from neighboring cells, where the summation runs over all the cells in contact with the ith cell. The second term on the right-hand side of Eq. 2.2a represents the forces associated with the interaction between the cell caps and the substrate/confinement wall. $\boldsymbol{\eta}_i$ is a random kick to the ith cell whose components are randomly drawn from the uniform distribution in the interval $[-10^{-6}\text{N}, 10^{-6}\text{N}]$. $\boldsymbol{M}_i$ in Eq. 2.2c is the torque on the cell with respect to the cell centroid. Finally, Eqs. 2.2a and 2.2b represent respectively the displacement and rotation of the cell in response to the forces and the torques. The constant ζ is a drag per unit length, which is assumed to be independent of the cell orientation. Possible origins of this drag are adhesive or frictional forces from the substrates, or from the ECM produced by cells during the colonization [62, 65, 66].

2.2 Continuum theory

Previous studies as well as our results from the molecular dynamics simulations have suggested that a growing colony exhibits orientational order but no positional order [38, 51, 59, 63, 65], hence is a nematic liquid crystal. In addition, we also find that cell growth collectively gives rise to a deviatoric stress reminiscing the famous *active nematics* [7, 75]. For this reason, we will use the continuum theory of active nematics to characterize a growing bacterial colony. Detailed discussion on the connections between growing bacterial colonies and active nematics will be shown in chapter 3. Here, we will introduce the general hydrodynamic equations of active nematics.

A nematic liquid crystal, or nematics, is a state of matter where the system shows orientational order but no positional order [76, 77]. This

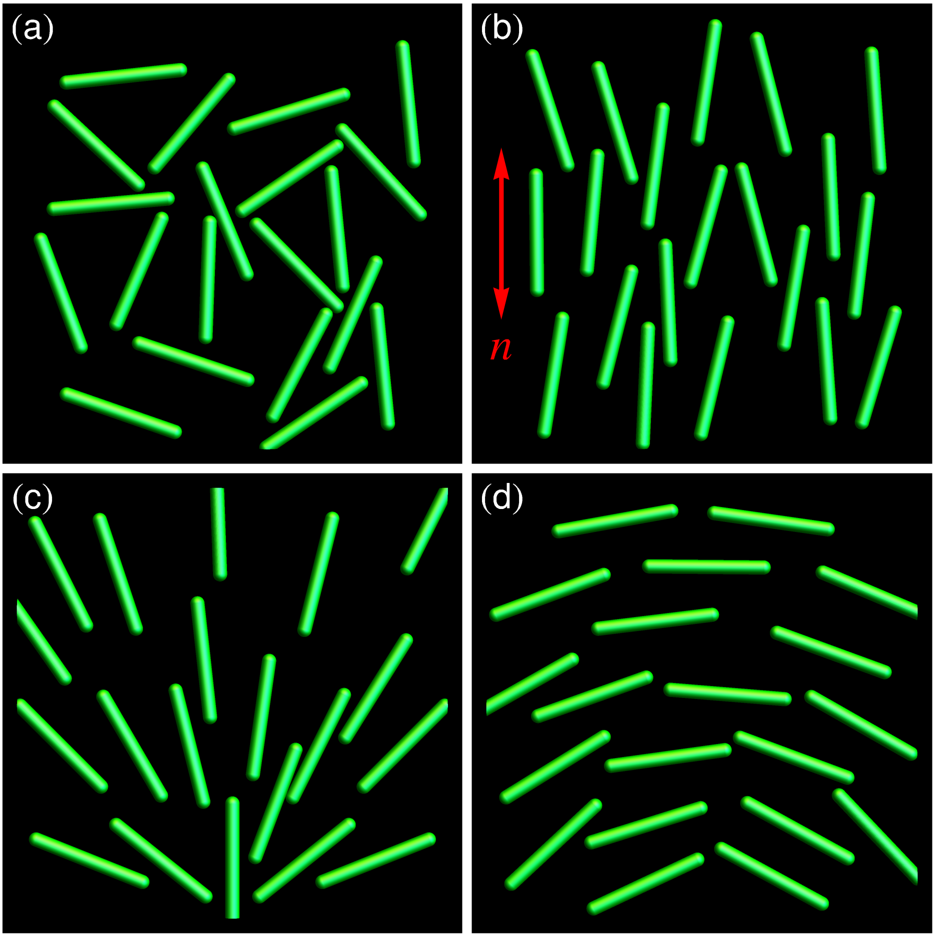

Figure 2.2. Orientational orders in nematic liquid crystals. (a) Isotropic state corresponding to an order parameter $S = 0$, and (b) highly aligned state with $S \approx 1$. The red arrow in (b) indicates the average orientation $\boldsymbol{n}$ of the particles. Note that $\boldsymbol{n} = -\boldsymbol{n}$. (c) Splay and (d) bending distortions of a two dimensional nematic liquid crystal.

orientational order can be driven either by excluded volume interactions between anisotropic-shaped particles, or anisotropic interactions between particles with arbitrary shape, and the resulting nematic phases are called lyotropic nematics and thermotropic nematics, respectively [76]. The orientational state of a nematics can be characterized by the so-called nematic order tensor $\boldsymbol{Q}$, which in a two-dimensional space is of the form:

$$\boldsymbol{Q} = S\left(\boldsymbol{nn} - \frac{1}{2}\boldsymbol{I}\right) . \tag{2.3}$$

In Eq. 2.3, S is called the nematic order parameter, which quantifies the degree of orientational order and has a value continuously distributed from $S = 0$ (no orientational order, Fig. 2.2a) to $S = 1$ (perfectly aligned, Fig. 2.2b). $\boldsymbol{n}$ is a unit vector representing the average orientation of particles. Note that $\boldsymbol{n}$ and $-\boldsymbol{n}$ represent the same orientational state. $\boldsymbol{I}$ is the identity matrix.

In addition to $\boldsymbol{Q}$, we can also use the density field ρ and the velocity field $\boldsymbol{v}$ to characterize the mechanical state of a nematic liquid crystal. The dynamics of these material fields are then governed by the following hydrodynamic equations [76–78]:

$$\frac{D\rho}{Dt} = \mathcal{D}\nabla^2\rho \, , \tag{2.4a}$$

$$\frac{D(\rho\boldsymbol{v})}{Dt} = \nabla\cdot\boldsymbol{\sigma} - \xi\rho\boldsymbol{v} \, , \tag{2.4b}$$

$$\frac{D\boldsymbol{Q}}{Dt} = \lambda S\boldsymbol{u} + \boldsymbol{Q}\cdot\boldsymbol{\omega} - \boldsymbol{\omega}\cdot\boldsymbol{Q} + \gamma^{-1}\boldsymbol{H} \, , \tag{2.4c}$$

where $D/Dt = \partial_t + \boldsymbol{v}\cdot\nabla + (\nabla\cdot\boldsymbol{v})$ is the material derivative. Equation 2.4a describes the conservation of mass of the particles, when transported across the system by convective currents. An additional diffusive term, with $\mathcal{D}$ a small diffusion coefficient, is introduced for regularization, i.e. to smooth the sharp gradient of ρ during the simulations. The particles' momentum density $\rho\boldsymbol{v}$ is subject to the internal elastic stresses $\boldsymbol{\sigma}$ as well as the frictional force $-\xi\rho\boldsymbol{v}$ from the substrate. The former can, in turn, be expressed as

$$\boldsymbol{\sigma} = -p\boldsymbol{I} - \lambda S\boldsymbol{H} + \boldsymbol{Q}\cdot\boldsymbol{H} - \boldsymbol{H}\cdot\boldsymbol{Q} \, , \tag{2.5}$$

where the first term represents the isotropic pressure of magnitude p. The remaining terms describe the elastic stresses arising from the aligning interactions between the particles. The molecular tensor field $\boldsymbol{H}$ in Eqs. 2.4c and 2.5 can be defined starting from the Landau–de Gennes free-energy density:

$$f_{\mathrm{LdG}} = \frac{1}{2} L_1 |\nabla \boldsymbol{Q}|^2 + \frac{1}{2} A_2 \mathrm{Tr} \boldsymbol{Q}^2 + \frac{1}{4} A_4 \left(\mathrm{Tr} \boldsymbol{Q}^2 \right)^2 , \tag{2.6}$$

as $\boldsymbol{H} = -\delta / \delta \boldsymbol{Q} \int \mathrm{d}A \, f_{\mathrm{LdG}}$. The first term in Eq. 2.6 promotes a homogeneous nematic order, for any gradient of the nematic tensor $\boldsymbol{Q}$, either from the order parameter S or from the director $\boldsymbol{n}$, will cost certain amount of free energy. In a two-dimensional nematics, possible distortions of $\boldsymbol{n}$ are splay (Fig. 2.2c) and bending (Fig. 2.2d), and $L_1 > 0$ is an orientational stiffness penalizing, in equal amounts of the two deformations. The last two terms in Eq. 2.6 describe a continuous phase transition between the isotropic ($S = 0$) and the nematic ($S > 0$) phases, where the boundary is set by functions A_2 and A_4 ($A_4 > 0$). At equilibrium, $\boldsymbol{H} = \boldsymbol{0}$ and we have

$$S = \begin{cases} 0, & \text{for } A_2 > 0, \\ \sqrt{-2A_2/A_4}, & \text{for } A_2 < 0. \end{cases} \tag{2.7}$$

If the nematic tensor $\boldsymbol{Q}$ deviates from the equilibrium configuration, $\boldsymbol{H} \neq \boldsymbol{0}$, and it will try to drive the $\boldsymbol{Q}$ tensor back to equilibrium through the following ways. First of all, a nonzero $\boldsymbol{H}$ will generate an orientational elastic stress as listed in the last three terms of Eq. 2.5. This stress can cause a material flow (the so-called backflow effect), which can then restore the $\boldsymbol{Q}$ field. Second, the molecular tensor $\boldsymbol{H}$ also plays the role of restoring torque which, according to the last term of Eq. 2.4c, can reorient the nematic tensor directly toward the equilibrium configuration, with a rotational viscosity γ. Finally, the particles also rotate as a consequence of the flow gradient. This effect is embodied in the first three terms of Eq. 2.4c, with $u_{ij} = (\partial_i v_j + \partial_j v_i - \delta_{ij} \nabla \cdot \boldsymbol{v})/2$ and $\omega_{ij} = (\partial_i v_j - \partial_j v_i)/2$ representing the strain rate and the vorticity tensor, respectively, and λ the flow-alignment parameter [78, 79].

Equations 2.4 can also be used to described active materials, and such systems are usually referred to as *active nematics* [7, 29, 75]. These have been successfully used in the past decade to describe a variety of active fluids, typically of biological origin, consisting of self-propelled or mutually propelled apolar building blocks, such as *in vitro* suspensions of microtubules and kinesin [29, 40, 80–86], microswimmers [41], and cellular monolayers [23, 75, 87]. Recently, attempts have been made to describe

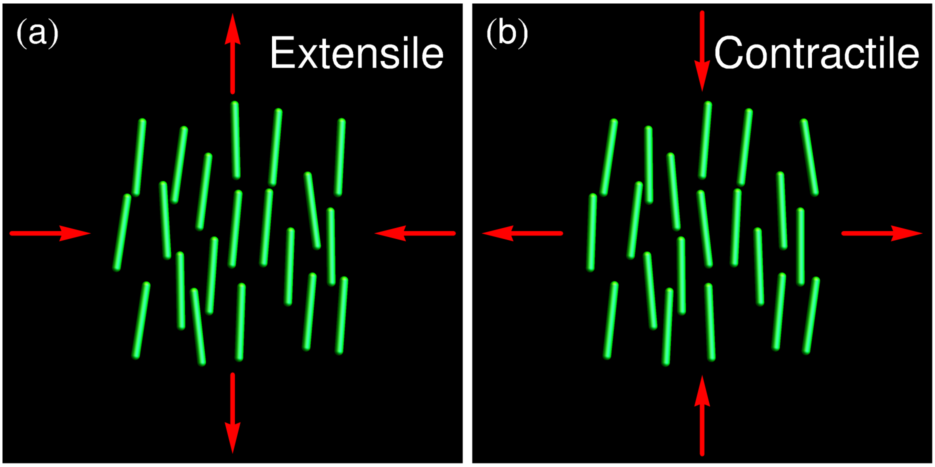

Figure 2.3. Sketches of (a) extensile and (b) contractile active stresses. The arrows show the stresses that the volume element exerts on the surroundings.

sessile bacteria, in the language of nematic liquid crystals [38, 63]. A common feature of these systems is that the activity of the cells or other building blocks collectively generates a deviatoric active stress [7, 29, 75]

$$\boldsymbol{\sigma}^{\mathrm{a}} = \alpha \boldsymbol{Q}. \tag{2.8}$$

Equation 2.8 describes a force dipole of a magnitude proportional to $|\alpha|$ and the nematic order parameter S, and with an axis parallel to the nematic director $\boldsymbol{n}$. The stress is called extensile if $\alpha < 0$ (Fig. 2.3a), and contractile if $\alpha > 0$ (Fig. 2.3b) [7, 29, 42, 75]. In the case of extensile active stress, the stress that a volume element exerts on its surroundings is extensile along the director $\boldsymbol{n}$ and contractile in the perpendicular direction. The contractile active stress has the same structure, but the forces are of the opposite directions. Experiments and simulations have shown that the active stress can drive the system far from thermal equilibrium, and can dramatically alter the dynamics of the system [7, 19, 29, 82, 86]. For example, the extensile active stress can destabilize a homogeneous director through bending, and lead to the proliferation of $\pm 1/2$ defect pairs [82]. It can also propel the $+1/2$ defects, and this can drive the flow into the turbulent region, creating the so-called *active turbulence* [7, 29, 86]. We shall see in the following chapters that the growth of bacteria will generate an extensile active stress and the colony can be well described by the hydrodynamics equations of active nematics with suitable modifications.