Cell-based models

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6.1 Introduction

The Myxobacteria fruiting body and the slug formed by starving Dictyostelium discoideum amoebae are two widely known examples of morphogenesis (see e.g. 16, 167, for reviews). These fascinating systems, which seem to teeter on the boundary between unicellular and multicellular modes of life, are initially composed of identical unicells that coordinate their actions using cell signaling (c-signaling in Myxobacteria and cAMP in D. discoideum). Despite their apparent simplicity, these systems display rich self-organizing behavior culminating in a spatially differentiated multi-cellular body. In this article we explore a little known system in the field of morphogenesis, which is simpler and much older than either amoebae or Myxobacteria: gliding filamentous cyanobacteria.

Cyanobacteria form a large and diverse phylum of prokaryotes that is chiefly defined by the ability to perform oxygen-producing photosynthesis
Chapter 6 – Modeling Reticulate Biofilms

Figure 6.1 – Comparison of in vitro reticulate patterns with ancient fenestrate microbialites from the 2.52-Ga Gamohaan Formation, South Africa. (A) Horizontal cross-section of the basal layer of a fenestrate microbialite. The arrow indicates the left edge of the tracing of the reticulate structure. Scale bar = 1 cm. (B) Experimental mat showing peaks and ridges at the same scale. The arrow indicates the right edge of the tracing. Note the similarities in structure between the ancient and experimental structures. Scale bar = 1 cm. Adapted from Shepard and Sumner (168) with permission from John Wiley & Sons Ltd.

(122). They play an extremely important role in the global eco-system by fixating carbon and nitrogen, providing a large part of the planet’s oxygen and providing sustenance to higher life forms. They can be planktonic or fixed in biofilms and benthic algal mats. They are also found in extreme environments such as deserts, the polar regions and hot springs.

Non-branching filamentous cyanobacteria are linear arrays of individual cells that form long and thin flexible trichomes (if the trichome has a sheath it is referred to as filament). Motile forms are capable of gliding, which occurs when the trichomes are attached to a (semi-)solid object (127). Recently, Shepard and Sumner (168) studied pattern formation in cultures of the filamentous cyanobacterium *Pseudanabaena*. They showed that in cultures with sufficient trichome density, the trichomes would organize within a matter of hours into a pattern of connected ridges defining a distinct polygonal reticulum (Fig. 6.1). They postulated that the patterns resulted solely from the random motility of the trichomes and that no form of signaling was required. They further speculated that reticulate patterns found in the fossil record may be due to a similar process.

These patterns, both fossil and extant, are important since they are associated with stromatolites – laminated sedimentary rock formed by the trapping and binding of sediment by filamentous cyanobacteria – which constitute the oldest evidence of life on Earth, the oldest dating to at least 3.4 Ga (billion
years ago; 169). In hot-springs in Yellowstone National Park and New Zealand, and in Antarctic lakes, reticulate patterns are found in the form of ridges connected to small cones or tepee-like structures (170, 171, 172). Fossilized forms of these extant patterns have been found in some of the oldest stromatolitic outcrops known (Fig. 6.1, ref 169) and the Yellowstone cones in particular have been proposed to be modern day analogues of much larger ancient stromatolites called *Conophyton* (170). Intriguingly, although filamentous cyanobacteria are ubiquitous in microbial mats found all over the planet (123), the formation of conical structures and reticulate mats appears to be uncommon. Identifying the specific physicochemical conditions and microorganism properties that lead microbial mats to form macroscopic structures can lead to a better understanding of the conditions on Earth at the dawn of life (173, 174, 175).

In this article we use a three-dimensional cell-based model to simulate the formation of reticulate patterns in cultures of filamentous gliding cyanobacteria. Our initial objective is limited in scope to testing and expanding on Shepard and Sumner’s hypotheses and studying the effect of varying the trichome parameters on pattern formation. The long-term goal, however, is to understand how the characteristics of stromatolite-building microorganisms and physicochemical conditions combine to form stromatolites both ancient and contemporary.

We used an extension of a previously published two-dimensional model of gliding filamentous cyanobacteria for our study (95). Three-dimensional cell-based models of bacteria have also been used for simulating Myxobacteria (e.g. 150, 151), including fruiting body formation (e.g. 19), *E. coli* (e.g. 53) and biofilms composed of coccoidal cells (e.g. 176). Systems of “elongated self-propelled rods” have also generated interest in physics for their self-alignment and swarming behavior, leading to a theoretical model of these systems analogous to the theory of liquid chrystals (152, 177). Many computational models of *D. discoideum* and *M. xanthus* fruiting body formation have been published, of which Marée and Hogeweg (40) and Sozinova et al. (18) are prime examples, respectively.
6.2 Methods

6.2.1 Biological Background

Cyanobacteria often form colonies with a multitude of forms, from square blocks to extremely long filamentous which may be branching or linear, straight or spiral (124). The cells within these colonies may be disk-shaped, cylindrical or even spherical, and cells may differentiate within a colony to perform a specific function, such as heterocysts specialized in nitrogen fixation.

Here we focus on non-branching filamentous forms of cyanobacteria, which have been found to drive interesting pattern formation (95, 170, 168, 132, 171). Pattern formation in these species is driven by gliding motility – a form of cell movement which occurs in the presence of a substrate and does not rely on any obvious external organ or change in cell shape (124, 125). Gliding in filamentous cyanobacteria appears to be enabled by a “slime jet” mechanism, similar to that found in Myxobacteria, in which the cells extrude a gel through a set of pores. The gel expands quickly as it hydrates providing a propulsion force (126, 127). Just as in the Myxobacteria, each cell has two sets of pores, one at each end. However since filamentous cyanobacteria are composed of an array of squat cells, and not a single elongated cell as the Myxobacteria, there are pores located all along the trichome at the cell junctions producing a tangential force along the length of the trichome. The cells appear to coordinate their gliding direction by an electrical potential that establishes polarity in the trichomes, and thus establishes a “head” and the “tail” (131). Based on the polarity, only one set of pores in each cell is active so the cells all push in the same direction. Trichomes usually reverse their polarity randomly with an average period on the order of minutes to hours (132, 133). However, depending on the incident light, cyanobacteria can adjust their reversal period so that they move into more favorable lighting conditions – a phenomenon called photomovement (132, 139, 140). Being photoautotrophs, cyanobacteria depend on light for survival yet intense radiation can inhibit photosynthesis or even kill the cells. Using a combination of photokinesis (gliding speed is a function of light intensity), phototaxis (gliding direction is a function of the incident light direction) and photophobia (gliding direction is a function of the temporal and spatial gradient of the light field), cyanobacteria can position themselves with great accuracy in
their environment (95, 132, 134, 135, 136, 137).

Photomovement was though to be essential for pattern formation in cyanobacterial mats and cultures (170). However, the formation of reticulate patterns in cultures of Pseudanabaena is likely to be a result of pure unbiased random gliding movement (168), since reticulates form in uniform lighting conditions (although self-shading may still have some effect). Therefore, we chose to ignore the effects of photomovement, and assume the trichomes reverse polarity with a constant frequency.

### 6.2.2 Model Geometry

We used a cell-based model of gliding bacteria in which trichomes are represented individually. Every trichome in the model has length \( L \) and diameter \( \Theta \) and is represented as a discrete chain of \( N \) edges of equal length \( l = L/N \) represented by \( N + 1 \) vertices. The dynamics of the system are inertialess and are given by the set of first order ordinary differential equations:

\[
\frac{dx_i}{dt} = \frac{1}{\zeta_i} \left( F_i^\parallel + \frac{1}{b} F_i^\perp \right) \tag{6.1}
\]

where \( x_i \) is the position of the vertex relative to the domain origin, \( \zeta_i \) is the drag experienced by the vertex and \( F_i^\parallel \) and \( F_i^\perp \) are the components of the total force acting on the vertex which are tangential and perpendicular to the trichome respectively, given by:

\[
F_i^\parallel = (F_i \cdot \hat{t}_i) \hat{t}_i \quad F_i^\perp = F_i - F_i^\parallel \tag{6.2}
\]

where \( \hat{t}_i \) is a unitary vector tangent to the trichome at vertex \( i \). For long and thin trichomes, \( b \approx 2 \) (178). Note that for simplicity we use a single index \( i \) on variables to denote the \( i \)-th of \( N + 1 \) vertices in a trichome centerline and omit an extra index \( f \) to denote the \( f \)-th trichome in the system, although the extra index is implied.

The total force is the sum of forces arising from bending, gliding and contact:

\[
F_i = \sum_{k=i-1}^{i+1} F^b(x_{k-1}, x_k, x_{k+1}) + \sum_{k \in \text{nd}_i} F^c(x_{i-1}, x_{i+1}, x_k, x_{k+1}) + F^g(x_{i-1}, x_i, x_{i+1}) \tag{6.3}
\]
6.2.3 Elasticity

To simulate trichome elasticity, we use the framework proposed by Bergou et al. (179) for modeling discrete elastic rods. Bergou et al.’s framework allows the trichomes to be represented by their centerlines using Cartesian coordinates, unlike other models which are based on curvature (e.g. 180). Using Cartesian coordinates facilitates coupling the elasticity equations with collisions and other processes.

Bergou et al.’s model assumes that the rod is inextensible, but allows for bending and torsion. Here we ignore torsion and enforce the inextensibility constraint by using the LINCS algorithm commonly used in molecular dynamics simulations (181). For a naturally straight and inextensible rod, the force on each vertex is given by the sum of up to three contributions (179):

\[ F_i = -\sum_{j=i-1}^{i+1} \frac{2\alpha}{l} (\nabla_i (\kappa b)_j)^T (\kappa b)_j \]

(6.4)

where \(\alpha\) is the bending modulus of the trichome, \(l\) is the length of the edges that compose the model trichome, and \((\kappa b)_j\) is called the discrete curvature binormal and is a vector perpendicular to the osculating plane between two consecutive edges whose magnitude is equal to the discrete curvature of the trichome at vertex \(j\):

\[ (\kappa b)_i = \frac{2e_i^{-1} \times e_i}{\|e_i^{-1}\|\|e_i\| + e_i^{-1} \cdot e_i} \]

(6.5)

The gradient of the curvature binormal is given by:

\[ \nabla_{i-1}(\kappa b)_i = \frac{2[e^i] + (\kappa b_i)(e^i)^T}{\|e_i^{-1}\|\|e_i\| + e_i^{-1} \cdot e_i} \]

(6.6)

\[ \nabla_{i+1}(\kappa b)_i = \frac{2[e_i^{-1}] + (\kappa b_i)(e_i^{-1})^T}{\|e_i^{-1}\|\|e_i\| + e_i^{-1} \cdot e_i} \]

(6.7)

\[ \nabla_i(\kappa b)_i = -\left(\nabla_{i-1} + \nabla_{i+1}\right)(\kappa b)_i \]

(6.8)

where \(e_i = x_{i+1} - x_i\), \([e]\) is a 3 \times 3 matrix such that \([e] \cdot x = e \times x\).

6.2.4 Gliding

Gliding in filamentous cyanobacteria is thought to be powered by a “slime-jet” mechanism. Thrust is generated by the extrusion of a dehydrated gel
through pores organized in rings at either end of every cell in the trichome (127). The expansion of the gel upon hydration generates a tangential force that propels the trichome forward. We make no assumption about the specific mechanism of gliding, we simply assume that a tangential force \( F_g \) is applied along the length of the trichome with a magnitude sufficient to propel the trichome at the target speed \( v_0 \). Gliding cyanobacteria also frequently reverse their gliding direction (132). We assume that each trichome has a polarization \( P_f \in \{-1, 1\} \) that determines the direction in which it is gliding. \( P_f \) is a stochastic process drawn at each simulation step for each trichome and is such that the trichomes reverse direction with an average frequency \( \omega \). The gliding force is given by:

\[
F^g_i = \zeta_i v_0 P_f \hat{t}_i
\]

where \( \hat{t}_i \) is a unitary vector tangent to the filament at the vertex \( x_i \):

\[
\hat{t}_i = \begin{cases} 
\frac{(x_{i+1} - x_i)}{\|x_{i+1} - x_i\|} & i = 0 \\
\frac{(x_{i+1} - x_{i-1})}{\|x_{i+1} - x_{i-1}\|} & 0 < i < N \\
\frac{(x_{i-1} - x_i)}{\|x_{i-1} - x_i\|} & i = N
\end{cases}
\]

### 6.2.5 Contact Interaction

In the model, the trichomes are modeled as three-dimensional articulated filaments, each of which is represented by a chain of edges that forms its centerline (Fig. 6.2(b)). The edges are of equal length, with each edge representing a piece of a trichome. Each piece is a straight cylinder with a spherical articulation at either end, forming a capsule shape (Fig. 6.2(a)). The trichomes only bend at the articulations and so when a trichome is bent only the articulations are deformed: they become rounded on one side and form a sharp angle on the other. The shape of the segments is determined by “cutting” the trichome at each vertex in the direction perpendicular to the tangent, except for the first and last vertices, which are assumed to be capped with semi-spheres (Fig. 6.2(b)). Thus the shape and orientation of a given trichome segment is a function of the edge’s vertices and tangent vectors.

Let \( \langle x_1, x_2 \rangle \) and \( \langle x_3, x_4 \rangle \) be any two edges in the model (Fig. 6.2(b)). We parameterize points on the first and second edges by the scalars \( a \) and \( b \) respectively such that any displacement vector between the edges can be
Chapter 6 – Modeling Reticulate Biofilms

(a)

(b)

(c)

(d)

(e)

(f)
given by:

\[ h(a, b) = (b x_4 + (1 - b) x_3) - (a x_2 + (1 - a) x_1) \quad 0 \leq a, b \leq 1 \] (6.11)

Segments interact by via steric and cohesion forces applied at discrete contact points \((a, b)\) where the edges come into close range (Fig. 6.2(c)). The forces resulting from an interaction between a pair of points \((a, b)\) are given by a Lennard-Jones type force field with strong repulsion for colliding edges and attraction for edges within a close vicinity:

\[ F^c = 12 \epsilon \left\{ \left( \frac{\Theta}{h} \right)^{13} - \left( \frac{\Theta}{h} \right)^7 \right\} \hat{h} \] (6.12)

where \( h = h(a, b) \), \( \Theta \) is the trichome diameter and \( \epsilon \) is the cohesion strength. The strong repulsion forces are meant to mimic the reaction forces of two rigid bodies colliding, whereas the attractive forces are meant to simulate the cohesion between trichomes due to the sticky slime they secrete while gliding. (To reduce numerical stiffness, the intensity of the repulsive forces was capped to a maximum value of \( R \).)

Since we represent each edge by its two vertices, any interaction force between edges must be translated into “equivalent” forces on the vertices by linear interpolation, assuming the segments are rigid. In terms of our

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Figure 6.2 (preceding page) – Modeling contact interactions between trichomes. (a) Individual segments are capsule-shaped and are defined parametrically, based on an edge. (b) Trichomes consist of a sequence of linked segments such that the underlying edges form a linear polyline. The shape of each segment is determined by “cutting” the segment at each of its vertices perpendicular to the local tangent. (c) Contact interactions are defined between pairs of edges. A simple linear parameterization \( h(a, b) \) is used for the interaction vector between the edges. (d) For any two non-colinear edges there is a plane that is parallel to both. If the edges cross each other when projected on this plane, then the interaction will be along the vector connecting the two edges at the interaction point. (e) For edges that do not cross, the four possible interactions between any vertex and the opposing edge are considered. (d) Only interactions that are “contained” within the domain of both segments are applied (only \( h_4 \) in this example.)
parametrization, a contact force $F^c$ between points $\langle a, b \rangle$ becomes:

\[
\begin{align*}
F_1^c &= (1 - a) F^c \\
F_2^c &= a F^c \\
F_3^c &= -(1 - b) F^c \\
F_4^c &= -b F^c
\end{align*}
\] (6.13)

The interpolation ensures the net force and net torque of the interaction forces are null.

To determine the interaction forces between two edges, first we calculate $a \times, b \times$ corresponding to the closest points between the lines the edges lie on (Fig. 6.2(d)):

\[
\begin{align*}
a \times &= \frac{\mathbf{r}_{34} \times \mathbf{r}_{13} \cdot \mathbf{r}_{12} - \mathbf{r}_{12} \cdot \mathbf{r}_{34} \times \mathbf{r}_{13} \cdot \mathbf{r}_{34}}{\mathbf{r}_{12} \cdot \mathbf{r}_{34} \times \mathbf{r}_{12} \cdot \mathbf{r}_{34} - \mathbf{r}_{12} \cdot \mathbf{r}_{12} \times \mathbf{r}_{34} \cdot \mathbf{r}_{34}} \\
b \times &= \frac{-\mathbf{r}_{12} \cdot \mathbf{r}_{34} \times \mathbf{r}_{13} \cdot \mathbf{r}_{12} - \mathbf{r}_{12} \cdot \mathbf{r}_{12} \times \mathbf{r}_{13} \cdot \mathbf{r}_{34}}{\mathbf{r}_{12} \cdot \mathbf{r}_{34} \times \mathbf{r}_{12} \cdot \mathbf{r}_{34} - \mathbf{r}_{12} \cdot \mathbf{r}_{12} \times \mathbf{r}_{34} \cdot \mathbf{r}_{34}}
\end{align*}
\] (6.14)

where $\mathbf{r}_{ij} = x_j - x_i$. If $0 < a \times, b \times < 1$, then the edges cross each other and the interaction vector between the crossing points $h \times = h(a \times, b \times)$ is the sole interaction considered.

If the edges do not cross, we consider the four interaction vectors between an edge and a vertex (Fig. 6.2(e)):

\[
\begin{align*}
h_1 &= h(g(x_1, x_2, x_3), 0) \\
h_2 &= h(g(x_1, x_2, x_4), 1) \\
h_3 &= h(0, g(x_3, x_4, x_1)) \\
h_4 &= h(1, g(x_3, x_4, x_2))
\end{align*}
\]

where $g(x_i, x_j, x_k)$ gives the closest point on the edge $\langle x_i, x_j \rangle$ to the point $x_k$:

\[
g(x_i, x_j, x_k) = \text{clamp} \left( \frac{\mathbf{r}_{ik} \cdot \mathbf{r}_{ij}}{\mathbf{r}_{ij} \cdot \mathbf{r}_{ij}} \right)
\] (6.15)

where

\[
\text{clamp}(x) = \begin{cases} 
1 & x > 1 \\
0 & x < 0 \\
x & \text{otherwise}
\end{cases}
\] (6.16)
Of the four pairs, in practice at most two will result in a non-null force.

Finally, only the interactions that are consistent with the shapes of the segments are considered. The shape conditions for each possible interaction vector are (Fig. 6.2(f)):

\[h_1: \quad r_{13} \cdot t_1 \geq 0 \text{ and } r_{23} \cdot t_2 < 0 \text{ and } (h_1 \cdot t_3 \leq 0 \text{ or } x_3 \text{ is an end vertex})\]

\[h_2: \quad r_{14} \cdot t_1 \geq 0 \text{ and } r_{24} \cdot t_2 < 0 \text{ and } (h_2 \cdot t_4 > 0 \text{ or } x_4 \text{ is an end vertex})\]

\[h_3: \quad r_{31} \cdot t_3 \geq 0 \text{ and } r_{41} \cdot t_4 < 0 \text{ and } (h_3 \cdot t_1 \leq 0 \text{ or } x_1 \text{ is an end vertex})\]

\[h_4: \quad r_{32} \cdot t_3 \geq 0 \text{ and } r_{42} \cdot t_4 < 0 \text{ and } (h_4 \cdot t_2 > 0 \text{ or } x_2 \text{ is an end vertex})\]

(6.17)

where the \(t_i\) are the tangent vectors as defined in Eq. 6.10.

### 6.2.6 Boundary and Initial Conditions

The trichomes are set in a cuboidal domain of dimensions \(W \times H \times D\). The dimensions are chosen such that the domain is shallow, i.e. \(H \ll W = D\). The boundaries perpendicular to the \(e_x, e_y\) directions (the sides) are periodic, but the boundaries perpendicular to the \(e_z\) direction (top and bottom) are "hard". Trichomes attempting to move above or below the top or bottom planes, respectively, are repulsed by a stiff reaction force. This force has not been included in Eq. 6.3 for the sake of succinctness, since it’s particulars are not important for the dynamics of the system.

The trichomes are initially set parallel to the \(xy\) plane with a random orientation and a random height. The model is then run with only the repulsive steric forces turned on, until the system reaches an equilibrium in which there are no overlaps between trichomes.

### 6.2.7 Nondimensionalization

In this section we nondimensionalize the model equations to determine the core set of model parameters. First we normalize the position and time variables using a characteristic length \(X\) and time \(T\) of our choosing:

\[X = x/L \quad \tau = t/T\]  

(6.18)

Then the equations are re-written in non-dimensional form:

\[
\frac{L}{T} \frac{dX}{d\tau} = \frac{1}{\zeta} \left( \frac{\alpha}{L^2} F^b + \zeta v_0 F^g + \epsilon F^c \right) \tag{6.19}
\]
where we have separated the parameters from the forces which are now only functions of the positions. The $1/L^2$ factor on the bending force is the result of substituting $X$ for $x$. Rearranging we have:

$$
\frac{dX}{d\tau} = \frac{T\alpha}{L^3\zeta} F^b + \frac{T\bar{v}_0}{L} F^g + \frac{Te}{L\zeta} F^c \tag{6.20}
$$

By defining the characteristic velocity $V = L/T$ and substituting we have

$$
\frac{dX}{d\tau} = \frac{\alpha}{VL^2\zeta} F^b + \frac{\bar{v}_0}{V} F^g + \frac{\epsilon}{V\zeta} F^c \tag{6.21}
$$

we choose the characteristic velocity and length of the system to be

$$
V = \bar{v}_0 \quad L = \sqrt{\frac{\alpha}{\bar{v}_0\zeta}} \tag{6.22}
$$

so that the first and second coefficients are reduced to 1, leaving a single non-dimensional parameter $\beta$:

$$
\frac{dX}{d\tau} = F^b + F^g + \beta F^c \tag{6.23}
$$

where $\beta = \epsilon/\zeta v_0$ is the ratio between the gliding and cohesion forces and is subsequently referred to as the cohesion strength.

### 6.2.8 Parameters

After making the equations of motion independent of scale we were left with a single non-dimensional parameter, $\beta$, in the equations. However the reversal frequency $\omega$ is another important parameter which is “hidden” in the stochastic reversals process. We denote the non-dimensional reversal frequency as $\Omega = \omega/T$. The system also has two non-dimensional parameters that characterize its configuration and subsequently the dynamics: the trichome density ($\rho$), measured as the volume fraction of the domain occupied by the trichomes, and the length-to-width ratio of the trichomes ($\gamma$). These four parameters form the core parameters which determine the model’s behavior.

The parameter values used in our simulations are based on Shepard and Sumner’s (168) experiments. The *Pseudanabaena* strain used in their study was on average 1.5 µm in diameter and up to “hundreds of micrometers long”.

We therefore assume a length-to-width ratio of $\gamma = 500$, which seems fairly common for filamentous cyanobacteria (see e.g. 156, 157), although reports rarely present a quantitative distribution of trichome lengths. The gliding speed was observed to be up to $1.3 \, \mu m \, s^{-1}$ for the *Pseudanabaena* trichomes and we take this value for $v_0$.

For the drag coefficient we follow Robinson et al., Lowe, Wiggins et al. (182, 178, 183) and use the analytical expression for the drag coefficient of a cylinder with $\gamma \rightarrow \infty$ and divide by the number of vertices per trichome:

$$\zeta = \frac{1}{N+1} \ln(2\gamma) + c$$

where $c$ is of order unity and depends on the shape of the filament, and $\mu$ is the viscosity of the fluid. We assume the drag coefficient is constant, ignoring the boundary effects of a trichome gliding very close to a wall, as well as hydrodynamical forces between trichomes. We assume $\mu = 1 \, \text{Pa} \, \text{s}$ (180) and $c = 1/2$ (182).

The bending modulus of the trichomes was first estimated using the data presented in Boal and Ng (184)'s study comparing ancient filamentous microfossils to extant cyanobacterial species. Although the authors only measure the persistence length $\xi$ of the trichomes, $\xi$ is directly proportional to the bending modulus $\alpha = k_B T \xi$. Therefore, with a persistence length of $480 \pm 50 \, \mu m$, the bending modulus of the *Pseudanabaena* strain they used would be approximately $1.94 \times 10^{-24} \, \text{Nm}^2$. This value is an order of magnitude smaller than the estimated bending modulus of *M. xanthus* (180), whose diameter is less than a fourth of *Pseudanabaena*'s and has a “patchy” peptidoglycan layer (185), making it relatively flexible.

How low is this value really? We can calculate a rough estimate of the Young’s modulus of the cell wall as measure of the material stiffness of the trichomes independent of their diameter. The bending modulus is given by $\alpha = YI$ where $Y$ is Young’s modulus and $I$ is the second moment of inertia of the cross section. Assuming that the bulk of the bending resistance lies in the cell wall, such that $I$ is equivalent to that of a hollow cylinder, we have $Y = 4\alpha/\pi(r^4 - (r-t)^4)$ where $r$ is the radius of the trichome and $t$ is the thickness of the cell wall. Assuming the cell wall thickness of *M. xanthus* is 2 nm (the lower bound for Gram-negative bacteria, 186) and its diameter is 0.5 µm, a rough estimate of the Young’s modulus of its cell wall is $\approx 3.1 \times 10^5 \, \text{Pa}$. This is two orders of magnitude lower than the benchmark
value of $1 \times 10^7$ Pa for the peptidoglycan layer of various bacteria (187, 188, and refs. therein), however we have already mentioned how Myxobacteria are typically more flexible than other bacteria. Performing the same calculation for *Pseudanabaena* assuming the cell wall is 10 nm thick (the lower bound for cyanobacteria bacteria, 186) and a diameter of 2.1 µm yields a Young’s modulus of just 54 Pa.

Although the deep constrictions between *Pseudanabaena*’s cells confer it greater flexibility, our initial estimate of the bending modulus seems to be unreasonably low. One possibility is that the bending observed in the trichomes in Boal and Ng’s study was not the sole consequence of random thermal fluctuations, as was assumed, and was exacerbated by cell motility and collisions with neighboring trichomes, thereby significantly underestimating the bending modulus via the relation $\alpha = \xi k_B T$. Also the strains used in Boal and Ng’s study seem to have been exceptionally fragile, as two of the three strains cultured had to be discarded because of extremely short trichome lengths, and the remaining strain produced very short trichomes of around 40 µm.

We resort to making an educated guess as to the true bending modulus of *Pseudanabaena*. Assuming that the trichomes are on the flexible side of the spectrum, we take $3.1 \times 10^5$ Pa as a lower bound on the Young’s modulus of the cell wall. Then, with a trichome diameter of 1.5 µm and taking 10 nm as the lower bound of cyanobacteria cell wall thickness, we arrive at $\alpha = 2 \times 10^{-21}$ Nm$^2$ for the bending modulus in our model.

The parameters of the model, as well as the values used, are summarized in Table 6.1.

### 6.2.9 Algorithms and Statistics

All simulations were performed using custom made C++ programs and executed on graphics processing units using the nVidia CUDA SDK version 4.0 provided by the nVidia Corporation. The “thrust” template library as well as the CURAND library for random number generation were used. Simulations were executed on an nVidia Tesla processing cards using single precision floating point arithmetic.

We experimented with the first order Euler method and the fifth-order Cash-Karp numerical methods, both with adaptive step-size control (52). Since we are not interested in the exact movement of individual trichomes, but rather the ensemble properties of the system, we decided to use the low
order Euler method with a target absolute accuracy of 0.1µm. We found that the simpler Euler method provided better performance when low accuracy is required.

To enforce the inextensibility of the trichomes we used the LINCS algorithm (181). The algorithm was applied after each unconstrained Euler step of the numerical method.

Contact interactions were handled by generating an interaction list consisting of pairs of edges within 2Θ distance of each other (our GPU implementation of this routine was inspired by the “particles” application by Simon Green, which is bundled with the CUDA SDK examples). The lists were reused until at least one vertex had moved Θ distance from its position when the previous list was generated. This effectively means that the Lennard-Jones function was truncated at $h = 2\Theta$, at which the force intensity is less than $<1\%$ of peak. However, a significant consequence of this computational optimization is that if two edges are outside of each other’s attractive range and are pushed into range through other forces, it is possible that the two edges will not feel an attractive force until the neighbor lists are updated. But once the update occurs the edges will always be subject to the attractive force until separated by other forces. In other words, a cohesive force is
guaranteed once edges are brought into close range, but the attractive force that may bring them together in the first place is not always consistent. We found this to be an acceptable compromise to achieve faster performance, since in reality the attractive force does not really exist, trichomes only cohere after they have come into contact.

Data analysis was performed using Mathematica 8. For each set of parameter values tested, five simulations with different random initial conditions were performed. The statistics shown in the Results section are the mean of the five simulations and the error is the standard deviation of the means for each system separately.

6.3 Results

There are four important parameters in the model: $\beta$ (gliding/cohesion ratio), $\Omega$ (reversal frequency), $\rho$ (trichome density) and $\gamma$ (trichome length/width ratio). We are mostly interested in the dynamics of very long trichomes, noting that there are many excellent studies on the dynamics of microorganisms with low $\gamma$ (e.g. 152, 177, 149, 189), and so we use a single high value of $\gamma$ in all the simulations (Table 6.1). For this initial study we vary the remaining three parameters one at a time. We are mostly interested in reproducing the experimental results of Shepard and Sumner (168), which showed robust formation of reticulate patterns in a wide range of conditions. We also seek to understand how the system parameters affect pattern formation.

6.3.1 Varying Cohesion Strength

In our initial round of simulations we tested the effect of cohesion strength on pattern formation. We tested values $\beta \in \{0, 0.25, 0.5, 1, 2.5, 5\}$. We only tested up to $\beta = 5$, since for higher values the trichomes would quickly form tight clumps and become immotile. For the values tested, three distinct patterns emerged from the simulations, as exemplified in Fig. 6.3. We first describe the patterns qualitatively and then apply various measures to justify our observations.

For $\beta = 0$ (Fig. 6.3a) the trichomes self-organized into broad, locally aligned streams within 12h. The topology of the streams appeared to be stable, even though trichomes are constantly passing in and out of them. The pattern is remarkable in that it is static in terms of its macroscopic struc-
6.3 – Results

Figure 6.3 – Varying $\beta$ (cohesion/gliding ratio) (a) $\beta = 0$, the trichomes organize into broad streams in which they become locally aligned. The streams narrow in places forming dense clusters of trichomes. Streams bend sharply in some places. The streams appear stable are $t = 12h$ (b) $\beta = 0.25$, the trichomes organize into thin parallel bands that are roughly globally aligned. The distribution of the trichomes appears stable at $t = 12h$. (c) $\beta = 1$, the trichomes form a chaotic network of thin bands. No discernible macroscopic pattern is observed. (d) $\beta = 5$, virtually all trichomes are banded. Bands are wavy and dynamic. A "dynamic pattern" of splitting and merging bands and loops is apparent.
ture, and yet is composed of constantly moving elements with no underlying structures or cues to enforce the pattern. At a few points the streams narrow and form dense constrictions (Fig. 6.4a), that seem to grow increasingly dense over time. Also the streams bend sharply at some places, forming distinct angles.

For $\beta = 0.25$ (Fig. 6.3b) a new pattern of thin elongated bands forms. The bands are all roughly parallel to each other, and so the system appears to be globally (nematically) aligned. The thickness of the bands varies, and the bands often branch and merge with other bands (Fig. 6.4b). The bands do seem to converge somewhat to form locally dense constrictions as before, but not to the same extent as with $\beta = 0$. After 12h, the patterns appear to be stable, and no rapid changes in the orientation or distribution of the trichomes is discerned.

For $\beta \in \{0.5, 1.0\}$ (Fig. 6.3c) the system becomes seemingly chaotic and any macroscopic pattern becomes difficult to discern. Thin bands still form, however they are now contorted and less aligned with neighboring bands. The bands branch frequently and merge with neighboring bands forming a complicated network with no obvious regular structure (Fig. 6.4c). Furthermore the network is not stable in time and is constantly changing topology as bands split or merge with neighboring bands. No stable macroscopic streams appear to form, as in the previous two cases.

For $\beta \in \{2.5, 5.0\}$ (Fig. 6.3d) cohesion is strong enough that virtually all the trichomes are located in a band. The bands are very contorted, yet form a coherent “wavy” pattern. The pattern is not static. The bands constantly split and merge with neighboring bands, changing the topology of the pattern. The bands tend to move perpendicularly to their long axis, increasing their curvature as they do. A notable feature of the system is the appearance of irregularly shaped loops (Fig. 6.4d). The loops tend to expand in size until they spontaneously dissolve, merge with a neighboring structure, or become “pinched” forming two loops.

To quantify the features of the system we used various different measures: global alignment, local alignment, local cluster size, trichome tangent correlation length, small sector alignment autocorrelation time and the small sector density frequency distribution. Global alignment ($\mathcal{G}$) was quantified
Figure 6.4 – Details of the simulations from Fig. 6.3. (a) $\beta = 0$. The broad streams narrow in some places forming dense spots. (b) $\beta = 0.25$, the thin parallel bands often branch and merge with others. (c) $\beta = 1$, bands become wavier and misaligned. (d) $\beta = 5$, irregular loops dynamically appear and disappear.
by calculating the length of the mean direction vector:

\[
G = \frac{1}{N_e} \left| \sum_{k}^{N_e} e^{i4\pi\theta_k} \right| \tag{6.25}
\]

where \(\theta_k\) is the angle of the \(k\)-th line edge in the model projected onto the xy plane. Local alignment (\(L\)) for each edge was calculated by taking the norm of the resultant of all the relative orientations of the edges within a within \(\Theta\) radius:

\[
L = \frac{1}{N_e} \left| \sum_{j}^{N_e} \sum_{k \in \text{nd}(j)} e^{i4\pi\theta_{jk}} \right| \tag{6.26}
\]

where \(\theta_{jk}\) is the angle between edges \(j\) and \(k\) (without projecting on the xy plane). The local cluster size (\(C\)) was calculated for each edge by counting the number of trichomes within a 2\(\Theta\) radius. The mean tangent correlation length (\(\xi\)) was calculated by first calculating the tangent autocorrelation function of each trichome

\[
\text{acf}_f(k) = \frac{1}{N - k} \sum_{i=1}^{N - k} e_{t,i} \cdot e_{t,i+k} \tag{6.27}
\]

where \(N\) is the number of edges per trichome and \(e_{f,i}\) is the direction of edge \(i\) of trichome \(f\). The mean tangent correlation length was then calculated by fitting the mean autocorrelation function \(\text{acf}(k)\) to the exponential function \(e^{-\xi k l}\) where \(l\) is the edge length.

The local alignment autocorrelation time (\(\tau\)) was calculated by binning the edges into a regular two-dimensional grid composed of 100 \(\times\) 100 \(\mu\)m sectors. For each sector the autocorrelation function of the nematic alignment within the sector was calculated over the last 2 hours of each simulation with \(\Delta t = 288\) s. The autocorrelation functions were then averaged over the whole domain and the autocorrelation time was then calculated in the same way as the tangent autocorrelation function. We also calculated the density frequency distribution of the sectors and compared it with the initial \(t = 0\) distribution. The measures are plotted in Fig. 6.5.

Of all the \(\beta\) values tested, \(\beta = 0.25\) showed the highest global alignment (Fig. 6.5a), consistent with the observations in Fig. 6.3. The \(\beta = 0\) simulations showed a high degree of alignment variability. Of the five simulations
6.3 – Results

Figure 6.5 – Quantifying system observables. Shaded areas correspond to the standard deviation of the means of five simulations. All simulations run for 12h except for $\beta = 5$ which was run for 8h. (a) Global alignment. Global alignment decreases with $\beta$, with the exception of $\beta = 0.25$, which showed the highest degree of alignment. (b) Local alignment. Weak cohesion leads to a high degree of local alignment as the trichomes align in streams and are straighter. Medium values disorganize the system and high values again lead to high local alignment as the trichomes aggregate into bands. (c) Local density increases with cohesion; $\beta = 0.25$ showing exceptional local density (d) Tangent correlation length decreases with $\beta$. For low $\beta$, trichomes become straighter with time. (e) Alignment autocorrelation time in $100 \times 100 \mu m$ sectors over 2 hours for each simulation. Red dot indicates the median value. Systems with little cohesion show much higher alignment correlation times than systems with significant cohesion. (f) Sector density frequency distribution. The initial uniform distribution is Gaussian with mean $\mu$ and standard deviation $\sigma$. Self-organization for $\beta \in \{0, 5\}$ is indicated by a skew in the density distribution as trichomes aggregate into patterns and depart from the uniform distribution. On the other hand, for $\beta = 1$ the system has a much more uniform distribution.
performed, one achieved a high degree of alignment $\approx 0.8$, but the remaining four simulations only reached $\sim 0.4$. For $\beta = 0.5$ global alignment was very weak, and for $\beta \in \{1, 2.5, 5\}$ the alignment of the edges was essentially random. For $\beta \in \{0, 0.25, 0.5\}$ global alignment was still increasing after 12h, and so it is possible that the system could achieve a high degree of alignment over an extended period of time.

As expected, the edges displayed a higher degree of local alignment than global alignment for all the $\beta$ tested (Fig. 6.5a). The highest degree of local alignment was observed for $\beta \in \{0, 0.25\}$. Local alignment dropped for $\beta = 0.5$, which is consistent with the observation that the system appears very chaotic for this value (Fig. 6.3c). Local alignment increases with $\beta$ for $\beta \in \{0.5, 1, 2.5, 5\}$ as more and more trichomes aggregate into bands (Fig. 6.3d).

Local density (Fig. 6.5c) increased proportionally with the relative cohesion strength as the trichomes aggregate into thicker bands. However $\beta = 0.25$ again showed exceptional behavior, with local density exceeding what would be expected from the general trend. The mean tangent correlation length (Fig. 6.5d) decreased with increasing $\beta$, consistent with what was observed in Fig. 6.3 where increasing $\beta$ resulted in wavier bands. However, for $\beta \in \{0, 0.25\}$, the mean tangent correlation length was increasing in time, indicating that the trichomes were becoming straighter.

The small sector alignment autocorrelation time is shown in Fig. 6.5e for each simulation. Higher values indicate longer persistence of the average direction in the sectors, which in turn indicates the stability of the stream pattern. The results show that systems with low cohesion have a much higher alignment autocorrelation, indicating the stability of these patterns relative to higher values of $\beta$. The sector density frequency distribution is shown in Fig. 6.5f. At $t = 0$, when the trichomes are scattered randomly, the density distribution is Gaussian with mean $\mu$ and standard deviation $\sigma$. Self-organization for $\beta \in \{0, 5\}$ is indicated by a skew in the density distribution as trichomes aggregate into streams/bands and depart from the Gaussian distribution. On the other hand, for $\beta = 1$ the system is less organized and the density distribution is closer to the initial Gaussian.

### 6.3.2 Domain Size, Density and Reversal Frequency

In this section we focus on the patterns obtained for $\beta = 0$ (Fig. 6.3a), which share qualitative similarities with the reticulate mats of Shepard and Sum-
The virtual trichomes form a network of steady streams within the same timeframe as the experiments. The size of the domain, however, is not sufficient to get a sense of the broader pattern. Therefore we performed a new set of simulations with $\beta = 0$ and a larger $1 \times 1$ cm domain. In addition, we decided to follow up on the observation that sufficient cell density was required to induce the reticulate pattern in the experiments. We performed simulations for four different densities $\rho \in \{0.3125, 0.625, 1.25, 2.5\}$.

The results are shown in Fig. 6.6.

For $\rho = 2.5\%$ (Fig. 6.6a), the same pattern as Fig. 6.3a emerges, but on a larger scale allowing one to get a better sense of the topology of the interconnected streams. The network formed by the streams does not have the regular structure of the patterns seen in Shepard and Sumner’s experiments. In those mats, the streams form a network constructed of sharply defined straight edges. In our simulations, the streams are neither straight nor sharp, but appear rather curvy and diffusive instead. Nevertheless, in both cases the topology of the streams forms and stabilizes within hours.

For all values of trichome density tested, the trichomes would form locally aligned streams. Decreasing trichome density had the effect of broadening the streams and making them less well defined, but streams are nevertheless clearly identifiable (Fig. 6.6b-d). In terms of the observables the system shows similar behavior as the previous simulations (data not shown). No simulation showed a high degree of global alignment, although the trichomes were locally aligned to a high degree. Local trichome density was directly proportional to the global density and the tangent correlation length was increasing in time for $\rho \in \{0.3125, 0.625, 1.25\}$, but seemed to stabilize for $\rho = 2.5\%$ at $\approx 4$ mm, unlike the previous simulations.

We also experimented with decreasing the reversal frequency from its typical value ($0.2$ min$^{-1}$). The results are shown in Fig. 6.7. Although streams are still apparent even for a relatively high reversal frequency (Fig. 6.7b), the pattern is clearly reinforced by more persistent movement.

### 6.3.3 Revisiting Low Cohesion

An interesting result of the first section was the exceptional behavior seen for $\beta = 0.25$. The virtual trichomes self-organized into thin, globally aligned bands. On the contrary, for $\beta \in \{0.5, 1\}$ the system appeared highly disorganized. We performed new simulations with $\beta \in \{0.125, 0.25, 0.5\}$ and a
Figure 6.6 – Simulations with no cohesion, a larger domain and variable density. (a) $\rho = 2.5\%$, trichomes form a network of streams. Streams are broad and ill-defined at some places, and narrow and focussed at others. (b) $\rho = 1.25\%$, streams still form but are less defined. (c) $\rho = 0.625\%$, the scale of the streams increases. (d) $\rho = 0.3125\%$, very broad and diffuse streams form.
Figure 6.7 – Varying the reversal frequency. $1 \times 1 \text{cm}$ domain. Streaming becomes more indistinct as the reversal frequency is increased. (a) $\omega = 1/100 \text{s}^{-1}$ (b) $\omega = 1/33 \text{s}^{-1}$ (c) $\omega = 1/11 \text{s}^{-1}$
larger 1 × 1 cm domain to see whether we might observe different patterns at a larger scale. The results are shown in Fig. 6.8.

For \( \beta = 0.125 \) (Fig. 6.8a) a stable pattern of multiple streams forms, however the streams are visibly broader and less numerous than in the cohesion-less case (Fig. 6.6a). For \( \beta = 0.25 \) (Fig. 6.8b) the streams are even broader and have a more homogenous appearance, since there are less constrictions and the streams describe more gentle curves. Clearly the global alignment in Fig. 6.3b is an artifact of the small domain size. Increasing \( \beta \) to 0.5, the stream pattern clearly begins to dissipate (Fig. 6.8c) as the virtual trichomes become more homogenously distributed in the domain.

### 6.4 Discussion

We have performed simulations of gliding filamentous cyanobacteria, which typically have a very high length-to-width ratio and are highly motile. We used a cell-based model in which each trichome is explicitly represented as a thin elastic rod. The virtual trichomes glide along their long axis at a fixed speed and periodically reverse direction. We also included a cohesion force, whose strength relative to the gliding force is given by the parameter \( \beta \). This was motivated by the fact that the trichomes exude copious amounts of EPS, which may act as a binding substance.

We found that for systems in which gliding overwhelms cohesion i.e. \( \beta \lesssim 0.5 \), the virtual trichomes self-organize into a stable pattern of streams. The streams observed formed a loose network of connected streams, each with a highly variable width, and a length on the order of a few micrometers. The topology of the streams was remarkably stable and the cohesion-less systems (\( \beta = 0 \)) displayed the sharpest stream patterns.

Increasing \( \beta \) caused the streams to become broader and smoother. For \( \beta \sim 1 \), in which the cohesion and gliding forces are roughly comparable, the system becomes a chaotic web of virtual trichomes and no evident pattern emerges. However, by increasing cohesion such that it overwhelms gliding, yet is not so strong as to reduce the system to dense clumps, \( 2.5 \lesssim \beta \lesssim 10 \), a fine mesh emerges, composed of thin bands of filaments. Unlike in the weak-cohesion systems, the topology of the mesh is dynamic.

The main objective of the model was to capture the behavior reported by Shepard and Sumner (168), who studied the formation of reticulate patterns in cultures of the filamentous cyanobacterium *Pseudanabaena*, and our
Figure 6.8 – Revisiting low-cohesion in a larger $1 \times 1\text{cm}$ domain. (a) $\beta = 0.125$, streaming occurs although the streams appear more diffuse. (b) $\beta = 0.25$, the global alignment seen in Fig. 6.3b is an artifact of the small domain size. The larger domain used here shows that a streaming pattern still occurs, except streams appear broader and smoother. (c) $\beta = 0.5$, the streaming pattern begins to fade as the system loses its large scale organization.
simulations bear similarity to their experimental results. As in Shepard and Sumner’s study, virtual trichomes were observed to align upon colliding and formed parallel clumps. In the experiments, a stable reticulate pattern would emerge within hours from an initially homogenous trichome mass inoculated on a submerged substratum. The patterns consisted of a network of ridges formed by densely packed filaments, each ridge being around 2 mm to 5 mm in length and 0.5 mm to 2 mm in height. Similarly, in the simulations we obtain a stable pattern of high density streams that surround low density areas, and the length and time scales of the patterns of both the simulations and experiments are similar. We also observe sharp angles in the system, which may be related to the polygonal forms traced by the ridges in the experiments. Our results indicated that a minimal system of self-propelled, very long trichomes is sufficient to produce a stable pattern consisting of multiple streams in which the trichomes are nematically aligned.

Despite the similarities described above, in the simulations the streams are less well defined than the ridges seen in Shepard and Sumner’s experiments, and the reticulate formed is irregular compared to the neat polygonal pattern seen in their results (Fig. 6.1). These differences may be due to the fact that we use a very shallow domain, just 7.5 µm deep, causing the pattern to be “squashed”. Another possibility is that the trichome density we used was too low. Shepard and Sumner (168) found that a minimum trichome density was required for ridges to form. In our simulations, we also found that increasing trichome density reinforces the stream pattern (Fig. 6.6), and it is reasonable to assume that increasing density further would help to consolidate the patterns.

Finally, it is also possible that our simple model is missing some essential characteristic of *Pseudanabaena*’s behavior. Shepard and Sumner (168) observed that the trichomes were capable of “bending laterally”, but it is not mentioned whether lateral bending has any effect on reticulate formation. *Oscillatoria terebriformis* trichomes exhibit both gliding and flexural movements and when the trichomes glide against each other they coil into rope-like structures (190, 191). A similar process may occur in the case of *Pseudanabaena*.

Another aspect is that we have assumed that our virtual trichomes may glide freely in the domain. In reality, gliding only occurs when trichomes are in contact with a solid surface, such as the substratum or another trichome, or embedded in a gel, such as agar. It is not clear whether the EPS produced
by the trichomes provides sufficient stiffness, or is produced in sufficient volume, to allow the trichome to glide freely. Restricting the virtual trichomes such that they may only glide when in contact with the substratum or another trichome may promote aggregation into ridges.

6.5 Conclusions

We have used a cell-based model to study the formation of reticulate patterns in cultures of *Pseudanabaena*. We have found that a minimal system of very long flexible trichomes capable of gliding motility is sufficient to produce stable patterns consisting of a coarse network of streams, however additional features of *Pseudanabaena* behavior, may be needed to fully explain the emergence of a polygonal reticulum in cultures. An ultrastructural characterization of the reticulum and a detailed and quantitative description of *Pseudanabaena* behavior would certainly add to our understanding of the pattern formation. On the other hand, larger simulations with a higher trichome density and a more realistic domain height may be sufficient to completely reproduce the reticulum.