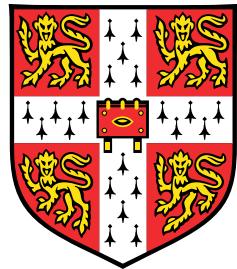


Geometry and shape inversion in *Choanoeca flexa*



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Declaration

I hereby declare that except where specific reference is made to the work of others, the contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other university. This thesis is my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text. This thesis contains fewer than 15,000 words including appendices, bibliography, footnotes, and tables.

Adam Konkol
August 2022

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Abstract

The newly discovered multicellular choanoflagella *Choanoeca flexa* and its cousin *Choanoeca perplexa* form colonies as curved sheets that can undergo a functional, light-triggered inversion. This change in orientation that allows the organism to reversibly switch between efficient swimming and feeding shapes provides an opportunity to study biological exploitation of geometry in an evolutionarily basal context. I sought to model the mechanics that produce this apparent bistability and the dynamics of the active transformation between the two states.

In this work, I approach the modeling problem from complementary continuous and discrete mechanics perspectives. Since radial expansion and contraction at a given latitude require azimuthal stretching and shrinking, a one-dimensional filament model does not capture the energetic challenges encountered by the sheet during the transition. Using energy functional variation, I write the sheet energy and write corresponding equilibrium shape equations, though they are too complex to treat analytically.

A discrete model for *C. flexa* colonies agrees with experimental evidence that collar stretching at sheet edges interferes in sheet inversion. Treating the organism as a crystal lattice defined by cells and cell-cell interactions, we recognise that the graph degree of cells substantially affects overall sheet curvature and the ability to invert. This finding agrees with the known geometric effects of topological defects in crystal lattices, where lattices must deform by buckling out of the plane to minimise their energy. Moreover, shape and inversion is dependent on the entire lattice topology. A lack or abundance of topological defects in a region may restrict sheet geometry on a large scale.

These results indicate that the topology of the cell-cell interface network must accommodate both colony bending and inversion. Moreover, it is clear that connection via collars is essential for sheet curvature by prescribing preferred mechanics at the cell body and shared interfaces. Future experimental work should image *C. flexa* flipping and look for any changes in connectivity that enable the transition.

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Nomenclature

Roman Symbols

- G Graph consisting of *C. flexa* cells as vertices and cell-cell interactions through collar microvilli as edges
- $g_{\mu\nu}$ First fundamental form of a surface
- G^* Graph consisting of collar microvillar interfaces between cells as edges and interface endpoints as vertices
- H Mean curvature of a surface, the mean of the two principal curvature $g_{\mu\nu}K^{\mu\nu}/2$. Preferred mean curvature (constant) is denoted H_0
- K Gaussian curvature of a surface, the determinant of the second fundamental form
 $\det K_\mu^\nu$
- $K_{\mu\nu}$ Second fundamental form of a surface
- \hat{n}_α Cell body orientation vector for cell α . Unit vector
- Pe Péclet number, the ratio between advective and diffusive transport in a fluid with specified flow velocity and length scale
- r_γ Vector position of vertex γ
- boundary cells cells with free collar microvilli

Greek Symbols

- α, β Indices used to index cell vertices in the discrete sheet description \mathfrak{G}
- γ Index for either cell (α, β) or collar (ρ, σ) vertices in the discrete sheet description \mathfrak{G}
- μ, ν Indices for a surface. $\mu, \nu \in \{1, 2\}$

- ϕ Denotes the angle between a cell orientation vector and cell-to-collar vector (equation (3.7)). Indexed $\phi_{(\alpha,\rho)}$ for cell α , collar ρ
- ψ Denotes half the angle between two collar interfaces (equation (3.11)). Indexed $\psi_{(\alpha,\beta:\rho,\sigma)}$ for cells α, β and mutual collar endpoints ρ, σ
- ρ, σ Indices used to index collar vertices in the discrete sheet description \mathfrak{G}

Subscripts

- $(\alpha, \beta : \rho, \sigma)$ Index for the interface given by collar vertices ρ, σ for cells α, β . The set of these indices is treated as ordered
- (α, ρ) Index for cell α and collar vertex $\rho \in \alpha$. The set of cell, collar pairs is treated as ordered

Other Symbols

- $\vec{\alpha\rho}$ Vector between vertices α and ρ , $\mathbf{r}_\rho - \mathbf{r}_\alpha$
- \mathcal{E} Total sheet energy. In the discrete sheet description, $\mathcal{E} = \mathcal{E}_\phi, \mathcal{E}_\psi, \mathcal{E}_{sp}$ is the sum of energies for angles ϕ and ψ and collar length, respectively

Acronyms / Abbreviations

- ECM Extracellular matrix

Chapter 1

Introduction

What physical driving factors led to the proliferation of multicellular life? Across all eukaryotic lineages, phylogenetic evidence suggests that multicellularity evolved independently several times [26]. Within just the volvocine algae, a common model family for studying the origins of multicellularity, it is believed that the transition to multicellularity and cooperation of distinct differentiated cells has repeatedly emerged independently [22]. Given the overwhelming success of multicellular life in the biosphere, we are led to hypothesize that common physical advantages promoted life to cooperate, differentiate, and delegate the responsibility of ensuring fitness for the sake of proliferation [18].

This question has led to flourishing recent interest in evolutionarily basal model eukaryotes, especially choanoflagellates, volvocine green algae, and sponges. Both the biological and physical properties of these groups make them especially favorable [17]: choanoflagellates have long been regarded as a close relative to the animal kingdom owing to their similarity to choanocytes in sponges [24], and the volvocine algae span a range of cell numbers per individual [27]. These organisms and their colonies are also large enough that they operate at high Péclet number ($Pe \sim 10^2$), the ratio between advective and diffusive transport, meaning that advection must contribute substantially to deriving food [54]. Consequently, these organisms must facilitate their food collection, unlike bacteria which rely on diffusion for nutrient transport [3].

1.1 Multicellularity

1.1.1 Origins of multicellularity

Before discussing eukaryotic multicellularity, I would be remiss in neglecting prokaryotic multicellularity [52]. Beyond the notorious biofilm formation, cyanobacteria (e.g. *Anabaena*) form chains of cells as filaments and some sporulating bacteria (e.g. Myxobacteria, *Step-tomyces*) form fruiting bodies [10]. These multicellular aggregates demonstrate complex population dynamics and emergent collective behavior from basic interactions [38, 61, 66]. Biofilms and filaments have phenotypically diverse and even differentiated regions that contribute to antibiotic resistance [56] or enable photosynthesis [16]. This differentiation can parallel germ-soma differentiation in eukaryotes by sacrificing individual division for colony persistence [37, 64]. Moreover, the extracellular matrix (ECM) of biofilms allows bacteria to exploit a tradeoff between environmental protection and growth by slowing diffusion from the exterior [40, 49]. The rest of this work centres around eukaryotic multicellularity, but it is valuable to keep in mind that several multicellular organisms have bacterial parallels.

The benefit of multicellularity for individual cells is not immediate. Michod and Nedelcu [43] argues that a key transition in the development of multicellularity is the transition away from selecting for single cell fitness to multicellular fitness. Indeed, while several pathways for multicellularity have been proposed, they share the idea that emergent group benefits through cooperation sufficiently outweigh individual sacrifice [44].

A major competing argument for the origin of multicellularity centers around physical size. Bonner [4] argues that size enables the advantages conferred by multicellularity, and scale itself comprises a spectrum of niches available for exploitation. An immediate consequence of an increase in size is the ability to avoid predation via phagocytosis. Stanley [55] argues that the emergence of heterotrophs, or organisms that derive energy by consuming others, led to rapid diversification and the rise of multicellularity through intense selective pressure. Boraas et al. [5] demonstrated in a laboratory culture that stable multicellularity emerged in the green alga *Chlorella vulgaris* when exposed to predators. Eight-cell colonies were observed to predominate, indicating that multicellularity emerged to balance colony survival as well as individual demands of each cell.

Multicellularity requires that resources are shared, and *cheat cells* which exploit this cooperation arise. Several have argued that exploitation and internal conflict may have facilitated evolution and differentiation that contributes to individuality of the whole organism [44, 48, 20, 18]. On the other hand, an altruistic strategy allows cells to cooperate and help

each other, increasing the success of the colony more than the sum of the individual cells [42, 19].

1.1.2 Benefits

Division of labour Differentiation of isolated cells

The organisms in the *Volvox* genus are well studied for being a primitive example of multicellularity and a shining beacon of functional geometric changes. These organisms attach their cells to one another using an extra-cellular matrix, as *S. rosetta* does as well (discussed later).

Generating flows With size increasing the Péclet number for an organism ($Pe = Lu/D$, where L is the length scale, u is flow velocity, and D is diffusion coefficient), multicellular organisms benefit more from advective nutrient transport as they become larger. The volvocine algae increase the proportion of advective flux as they increase in size by generating a thin boundary layer in solute concentration, in turn facilitating long-range nutrient transport [53].

The advantages of multicellularity for choanoflagellate flows are yet unclear. Kirkegaard and Goldstein [28] modeled that the best flows through a collar of microvilli are generated when cells swim independently, aligning with the idea that unicellular swimming is optimal feeding [41]. Rosette choanoflagellate colonies are even worse at generating flows through collars, as their flagella all point in different directions.

For the colony geometries studied in this work, however, we may compare to sponge choanocyte chambers and the substantial flows they are capable of producing. Sponges drive flow using choanocytes, which are morphologically and functionally similar to choanoflagellates despite being animal cells [31, 9]. By organising into spherical chambers, sponges are able to efficiently generate flows and chain together pumps to drive macroscopic flow [2]. Deriving from the first animals, sponges bring the evolutionary conclusions from these models for basic multicellularity closer to increasingly complex animals [46].

1.2 Choanoflagellates

Choanoflagellates are aquatic heterotrophs that feed in their motile stage by driving flows through a collar of microvilli using a single apical flagellum [25]. Choanoflagellates are considered the genetically closest group to metazoans [9], though the association between choanoflagellates and animals predates studies using molecular phylogeny. James-Clark [24]

first compared choanoflagellate morphology with the choanocytes in sponge choanocyte chamber. Morphological similarities between choanocytes and protists led to the belief that the two emerged from a common ancestor [12, 59].

Sponge choanocytes and choanoflagellate morphology bear substantial resemblance to each other in that both collars and apical flagella. However, choanoflagellate cells always have collar filaments, while choanocytes develop them through differentiation [39]. Choanoflagellate glycocalyx tends to surround the cell body, while sponges use it to form a web with the collar microvilli [36].

Choanoflagellates are increasingly studied as a model for understanding how multicellular animal life emerged. Much of the recent work on choanoflagellates has been done with *Salpingoeca rosetta*, a model for the development of multicellularity for its propensity to form multicellular rosettes bound by an extracellular matrix (ECM). Based on *S. rosetta* studies, our understanding of choanoflagellate colony formation is that single cells initiate colonies by cell division, rather than aggregation [15]. Additionally, choanoflagellate colony morphology appears to be dictated by the ECM material properties, producing a variety of structures attached at cell bodies [32].

The reasons for colony formation (i.e. multicellularity) in choanoflagellates are unclear. Kirkegaard and Goldstein [28] found that collared choanoflagellates drive the most flow through their collars by swimming fastest, which occurs when colonies are unicellular. Despite the complicated feeding geometry resulting from bacterial capture at the collar, this result is consistent with the result that optimal feeding aligns with optimal feeding for small swimmers [41]. Hence, rosette formation in *S. rosetta* or adhesion in any choanoflagellate is expected to benefit the feeding rates of individual cells. Evolutionary causes of colony formation are obscured further by strong recent evidence that rosette formation in *S. rosetta* is induced by chemical cues from bacteria [1, 65].

1.3 *Choanoeca flexa*

Brunet et al. [7] describe a newly discovered choanoflagellate, *Choanoeca flexa*, which lives and feeds in aquatic environments (figure 1.1). Here, I describe the relevant properties and characteristics of these cells and their colonies for modeling its structure and behavior. All descriptions of *C. flexa* proceed from Brunet et al. [7] and private communications with the authors.

C. flexa is an aquatic colonial choanoflagellate that forms sheets on the order of 100 μm in diameter (figure 1.1). Each cell in a colony consists of a cell body ($\sim 4\mu\text{m}$), microvillar

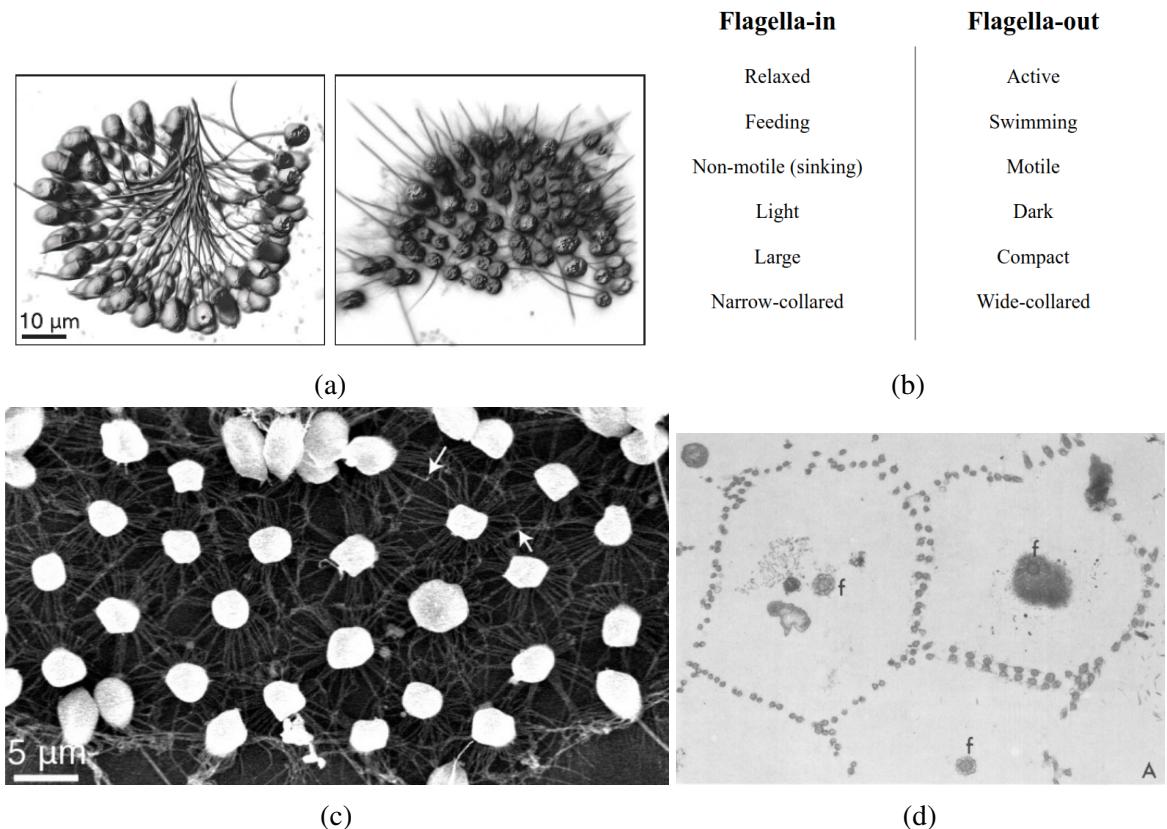


Fig. 1.1 Overview of *C. flexa* colonies of collared choanoflagellate cells. (1.1a) Images of the two conformations observed, flagella-in and flagella-out. (1.1b) Summary of the two states of *C. flexa* colonies. The transition from flagella-in to flagella-out is induced naturally by darkness, and the reverse transition is induced by the reintroduction of light [7]. (1.1c) Scanning electron micrograph in the tangential plane of a *C. flexa* sheet. Arrows indicate collar-collar contacts. (1.1d) Transmission electron micrograph of collar interactions between neighboring cells in a *C. perplexa* colony. f: flagella. From Leadbeater [35] with permission. figures 1.1a and 1.1c from Brunet et al. [7]. Reprinted with permission from AAAS.

collar ($\sim 10\mu\text{m}$ length), and apical flagellum at the collar centre. All observed sheets have had flagella facing in the same direction, giving the sheet two distinct sides. Cells attach to each other through their collar microvilli, and in contrast to a colonial flagellate like *S. rosetta*, there is no evidence for an extracellular matrix holding cells together in *Choanoeca* [35, 7]. Collar microvilli are distinct and colony cells demonstrated no intercellular cytoplasmic bridges (contrast with *i.e.* *Volvox*), with cells detaching from each other upon treatment with calcium [6]. By comparison with division in *C. perplexa*, colony cells are expected to undergo cell division with temporary incomplete replication, where the pair of daughter cells is attached by some shared collar microvilli (figure 1.2). Colonies are believed to occasionally fragment to separate completely and multiply [35].

C. flexa also exhibits a sedentary, unicellular form that adheres to surfaces via a stalk (*theca*) without a flagellum, as in *C. perplexa*. Our understanding of cell division in *Choanoeca* emerges from the thecate form, which leaves one thecate daughter cell and another motile, flagellated cell. Division begins with the generation of a flagellum by the thecate cell and proceed with protoplasm division with incomplete separation at collar microvilli (figure 1.2) [14, 34]. The remainder of this work concerns the colonial form of *C. flexa* rather than the thecate or unicellular motile forms. While we do not have observations on cell replication in the colonial phase, *S. rosetta* gives a suggestion that colonial choanoflagellates do not coordinate their cell division [15].

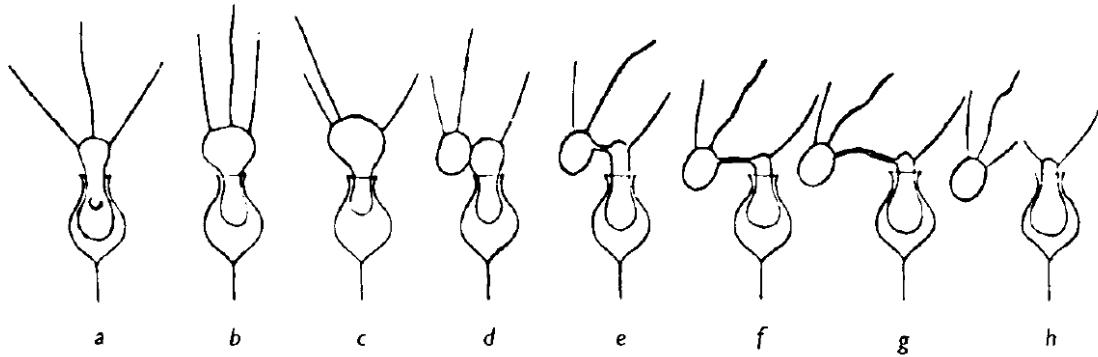


Fig. 1.2 Illustrations of stages of division in the sessile form of *C. perplexa*. Reproduced from Leadbeater [34] based on Ellis [14] with permission from Cambridge University Press.

1.3.1 Sheet inversion

Choanoeca has recently sparked renewed interest as a result of the characterisation of rapid light-regulated inversion in colonies, which causes cell sheets to change orientation from

pointing flagella-in to flagella-out [7]. The inversion is understood to result from contraction of an actomyosin ring at the apical end of colony cells, which results in collar microvilli flaring out. This result is consistent with a description of contraction the cell apex with changes in collar angle for *C. perplexa* cells [34]. Sheet inversion takes ~ 10 s. Notably, deviations in *C. perplexa* collar angle (between $10^\circ - 90^\circ$ from the apicobasal axis) were described in Ellis [14], and Leadbeater [35] described colonies now understood to be *C. perplexa* undergoing inversion.¹ Collar stiffness and the intrinsic curvature in collars facilitates a clear preference in sheet curvature. Collars in thecate cells have been described as flaccid [34], suggesting that an increase in collar stiffness is essential to the transition to colony-forming cells.

Brunet et al. [7] identified several factors that contribute or prohibit sheet inversion. It is believed that, in their natural environment, sheet inversion from flagella-in to flagella-out is triggered by darkness. In the inverted (flagella-out) state that occurs in darkness, individual cells demonstrated contraction of an apical actomyosin ring. The contraction results in collar microvilli flaring out: relative to the apicobasal axis measured at the base of the flagellum, the median collar microvillus angle moved out from $\sim 35^\circ$ to $\sim 50^\circ$. This increase in angle is largely the result of collar microvilli straightening out: in light, single colony-cells' collars curve to align with the apicobasal axis after emerging from the apical ends of cells. Cell sheet area decreases significantly since cell bodies are in contact in the inverted state [6]. These properties are summarised in figure 1.1b.

The transition to the flagella-out state results in a significant increase in swimming speed [7]. In contrast, flagella-in sheets are non-motile to the extent that they typically sink. The lack of mobility is compensated by an increase in cells phagocytosed by several times: flagella-in sheets are substantially more effective at driving flow towards the collars, where individual cells feed. Increased motility in the dark-induced state and accumulation in regions with light facilitates a primitive form of phototaxis.

Sheet inversion is rapid and reversible, allowing colonies the flexibility to convert when given suitable environmental cues.

1.4 Thesis overview

C. flexa provides an opportunity to study geometric changes through a simple, uncoordinated mechanism in an evolutionarily basal context. In this thesis, I present several approaches for

¹The latter attributes inversion to a reversal of flagellum rotation, though this explanation is unlikely given the more recent evidence for *C. flexa* [7].

modeling *C. flexa* colony sheets from a mechanics perspective. I discuss relevant models from continuous mechanics and find that the simultaneous stretching and compression in several collar microvilli must be taken into account to appropriately model shape and dynamics. I review shape equations derived from variation of surface energies defined in terms of curvatures and derive an energy function for a continuous description of *C. flexa* sheets.

Due to the complexity of the continuous model, I develop a more tractable discrete model based on lessons from the continuous description. Collar microvilli are simplified from filaments to elastic rods, which permits a simple energy function to be defined in terms quadratic potentials on cell-collar distances and angles defined by connecting cells and collars. The model consists of two parameters: equilibrium angles ϕ_0 (preferred angle from the collar base to the apicobasal axis) and ψ_0 (preferred collar-collar contact angle). I take the gradient of the energy with respect to cell and collar coordinate vectors as well as the apicobasal axis vectors of all cells to solve the forces acting on all free variables in the system. I numerically integrate the forces on all spatial coordinates and torques on cell axes to study dynamics of cell sheets and study their mechanical equilibria.

My discrete model for *C. flexa* colonies successfully models inversion in small sheets consisting of few cells. In larger sheets, conformational changes are hindered by rings of cells which cannot undergo the requisite stretching or compression required for inversion or folding. When sheets are already curved with flagella pointing in or out, the inability to sufficiently stretch at the boundary prevents sheets from inverting and causes the cells on the sheet interior to experience substantial stress. By framing my discrete model of *C. flexa* using graph theory, I identify that the topology of the cell-cell connection lattice determines a sheet's ability to bend without buckling or overstretching. This finding and my structural results compare well with the understanding of geometric effects of topological defects in crystal lattices.

The model I present makes it possible to identify regions where equilibrium angles ϕ_0 and ψ_0 give minimal energy structures in the flagella-in and -out conformations. For values that facilitate a flagella-out structure, the flagella-out structure is lower in energy than the flagella-in structure when sheet size prevents inversion as expected. I argue that the greater amount of time required for larger sheets to invert is the result of a larger energetic barrier or smaller net internal forces, rather than greater hydrodynamic damping through drag. This effect from topological constraints at the sheet boundary explains the rapid contraction and slow inversion observed in large sheets in [7]. My model supports the hypothesis that *C. flexa* sheets are able to invert as a result of extreme stretching at sheet boundaries or topological changes through collar-collar linkages temporarily breaking.

Chapter 2

Continuous model

Sheets of *C. flexa*, despite being discretely made up of individual cells, appear to take on curvature when looked at as a whole. In an effort to develop an analytically tractable model for sheets consisting of many cells and avoid building a detailed network topology, I approximate here sheets of *C. flexa* using continuous functions.

I begin by developing a one-dimensional filament model with inversion dynamics, which demonstrates that azimuthal stretching is key to understanding the flipping process observed in Brunet et al. [7]. I proceed to describe a method for approximating sheets of *C. flexa* with two-dimensional surfaces and relate the collar-opening angle ϕ and collar-collar contact angle ψ to surface curvature. I write an expression for the sheet energy and vary it to derive a shape equation.

2.1 One-dimensional model

We are interested in the problem of *Choaneca flexa* inversion. To build intuition, consider a chain of cells connected by their collar filaments like beads on a string. Supposing there are sufficiently many cells that the length contributed to the chain of cells by a single cell is small relative to the total length, we approximate the filament with a continuous function $\vec{r}(s)$ parameterised by arclength s . If the filament has preferred curvature κ_0 , then the bending energy functional ε is given by

$$\varepsilon[\vec{r}(s)] = \frac{1}{2}A \int (\kappa - \kappa_0)^2 ds,$$

where the curvature κ is deduced from $\vec{r}(s)$ and A is the bending modulus.

If the filament is short relative to the characteristic bending length scale, we express the problem in the Lange representation by writing $\vec{r}(s) = (x, h(x))$ for a height function $h(x)$. The resulting energy is written in terms of the prescribed (signed) curvature H_0 ,

$$\epsilon[\vec{h}(x)] = \frac{1}{2}A \int_0^L (h_{xx} - H_0)^2 dx. \quad (2.1)$$

Since the cells in this beads-on-a-chain description consist of large spheres connected by thin filaments, we postulate that the filament experiences isotropic drag with coefficient ζ in a viscous medium. As a result, we describe the dynamics with a functional derivative of the energy with respect to the changing height,

$$\begin{aligned} \zeta \vec{r}_t &= -\frac{\delta \epsilon}{\delta \vec{r}} \\ \zeta h_t &= -\frac{\delta \epsilon}{\delta h}. \end{aligned} \quad (2.2)$$

Taking the functional derivative of equation 2.1, we find the energy change

$$\delta \epsilon = A(h_{xx} - H_0)\delta h_x|_0^L - Ah_{3x}\delta h|_0^L + A \int h_{4x}\delta h ds \quad (2.3)$$

in terms of the boundary conditions of h .

For free boundary conditions (force- and torque-free edges) $h_{xx}(0, L) = 0 = h_{3x}(0, L)$, the boundary terms in equation 2.3 vanish and we are left with the equation of motion

$$\zeta h_t = -Ah_{4x}. \quad (2.4)$$

Equation 2.4 is nondimensionalised by re-expressing x as x/L and t as $t/(\frac{\zeta L^4}{A})$ (the labels x, t, h, H_0 are left unchanged for readability) to derive $h_t = -h_{4x}$ with boundary conditions $h_{xx}(0, 1) = 0 = h_{3x}(0, L)$. It is clear that the ground state of equation 2.1 is given by a quadratic height function with quadratic term $\frac{1}{2}H_0x^2$. Let $h_*(x) = -\frac{1}{2}H_0(x - \frac{1}{2})^2 + \frac{1}{8}H_0$ be one such ground state, and suppose $h(x, 0) = -h_*(x)$. Note that the filament in $h(x, 0)$ is not in the ground state since the curvature is given by H_0 .

The dynamics of the displacement $g(x, t) = h(x, t) - h_*(x)$ is given by $g_t = g_{4x}$. If $g(x, t) = e^{-\sigma t}f(x)$ for some $f(x)$ and eigenvalue σ , we obtain the ordinary boundary value problem

$$\frac{d^4 f}{dx^4} = \sigma f \quad \begin{cases} f''(0, 1) = 0 \\ f'''(0, 1) = 0. \end{cases} \quad (2.5)$$

It is clear that the general solution of f is $A \sin kx + B \cos kx + D \sinh kx + E \cosh kx$ [30]. As in Wiggins et al. [63], the derivatives $f''(0) = f'''(0) = 0$ give $A = D, B = E$. Moreover, the eigenvalues $\sigma = k^4$ are given by the sequence of solutions k_n to

$$\cos k - \frac{1}{\cosh k} = 0. \quad (2.6)$$

Equation 2.6 is plotted in Figure 2.1a along with the positions of the solutions k_n as solved numerically. The solution $k_0 = 0$ is omitted because it contributes a constant term to $h(x, t)$ that does not evolve in time. The eigenfunctions $w_n(x)$ with eigenvalues k_n^4 are normalized on the interval $[0, 1]$ numerically, and the ratio A/B is given by $(\sinh k - \sin(k)) / (\cosh k - \cos k)$. The first five eigenfunctions are shown in Figure 2.1b.

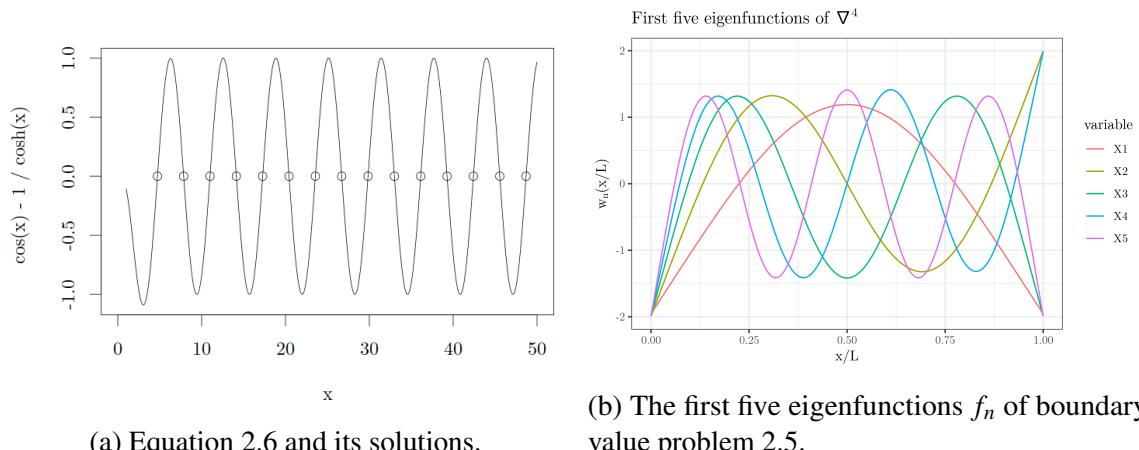


Fig. 2.1 Solving the boundary value problem in equation 2.5

Letting $f(x) = \sum_{n=1}^{\infty} a_n f_n(x)$, we get that $a_n = \int_0^1 g(x, 0) f_n(x) dx$, and the complete dynamics of the height function are given by

$$h(x, t) = h_*(x) + g(x) = h_*(x) + \sum_{n=1}^{\infty} a_n e^{k_n^4 t} f_n(x). \quad (2.7)$$

In practice, only the solutions k_n to equation 2.6 shown in Figure 2.1a are used, since the approximation to $h(x, 0)$ is close and higher k_n result in precision errors when calculating $\cosh kx$ and $\sinh kx$.

For the initial conditions given previously, the time evolution of $h(x, t)$ is shown in Figure 2.2. Immediately, we notice that the filament changes shape extremely quickly, and the timescale $\zeta L^4/A$ must be extremely large to produce inversion at the order of 10 sec as observed by Brunet et al. [7]. Using Stokes' law $\zeta = 6\pi\mu R$ for dynamic viscosity μ (about 1 kg/m/sec) [57] and cell radius $R \approx 1 \times 10^{-6}$ m [7], a chain of 100 cells each contributing 5×10^{-6} m length would require energy constant about

Besides the issue of timescale, the dynamics in figure 2.2 also fail to capture the *rolling over* phenomenon observed at the edges of large sheets [7], where a wave of changing curvature propagates from the edge of the sheet towards the centre. While the edges show a slight rim around $t \approx 9 \times 10^{-4}$, the effect is not as pronounced as in *C. flexa* inversion and does not occur at the initiation of the transition. A possible explanation for this effect in the cell sheets is that the collars resist compression or stretching. The representation used here, on the other hand, does not penalise filament compression.

As the arclength decreases substantially during the transition in figure 2.2 without affecting its dynamics, it is clear that collar extension and compression are essential to describing appropriate dynamics. Moreover, the timescales between this one-dimensional model and those observed experimentally being as misaligned as they are indicates that some energetic barrier to inversion is missing in the above description. We identify here the key deficiency of the one-dimensional description of *C. flexa* sheets, which is that compression and extension, especially in the azimuthal direction, are essential to understand the sheets' bistable nature.

2.2 Surface approximation

The simplified dynamics that we get from the Mange representation lack energetic costs from the compression and extension that come with deforming a two dimensional surface. Here, I evaluate two continuous approaches to describing the sheet as a continuous surface that approximates the positions of collar-collar interfaces. Since bending and stretching are coupled in *C. flexa* sheets, I write a single energy function that encompasses both.

The resulting energy function is found to be too challenging to approach analytically and motivates chapter 3 to describe the *C. flexa* sheet as a discrete system.

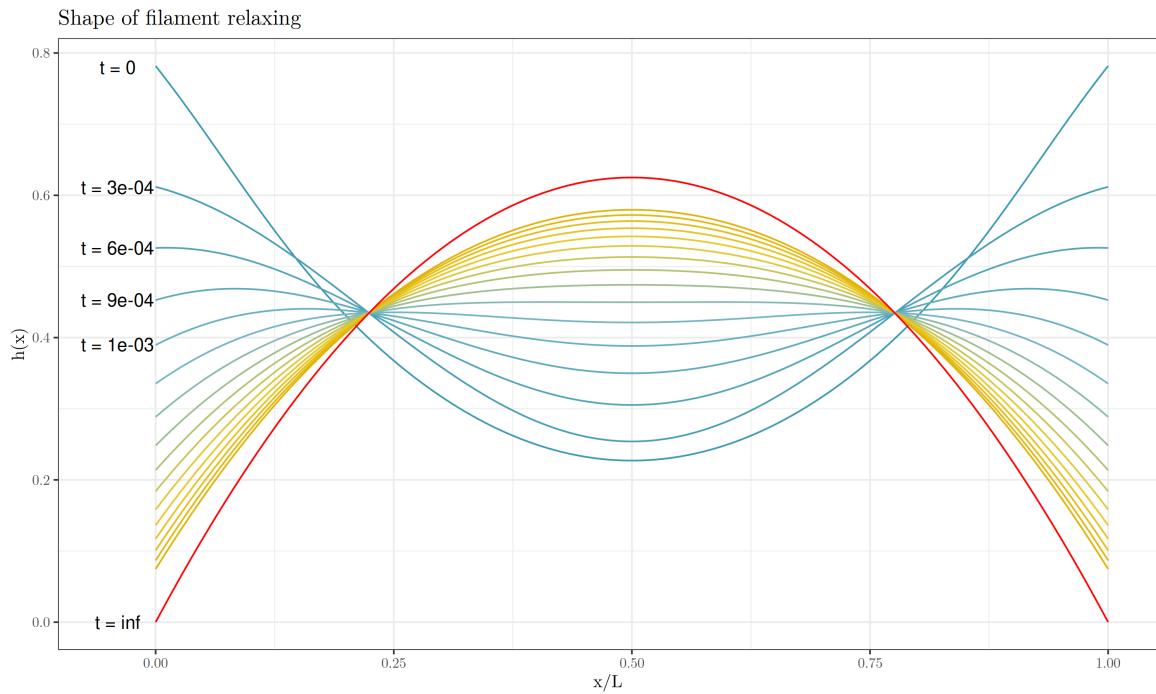


Fig. 2.2 Time evolution of a one-dimensional filament which switches prescribed curvature sign instantaneously. Time and length are given in dimensionless units defined by the length L , bending modulus A , and drag coefficient ζ . The time unlabeled time intervals continue sequentially in intervals of $\Delta t = 3 \times 10^{-4}$.

2.2.1 H and collar connection angle

Before getting into the continuous sheet problem, it is worth describing the two degrees of freedom that our collar connections afford. The collar makes an angle ϕ between the vector pointing directly out of the cell and the vector between the cell and its collar boundary with the next cell. Additionally, there is an angle between the collars of two adjacent cells ψ . The latter results in the sheet's curvature, so we want to relate it to mean curvature H or preferred curvature H_0 .

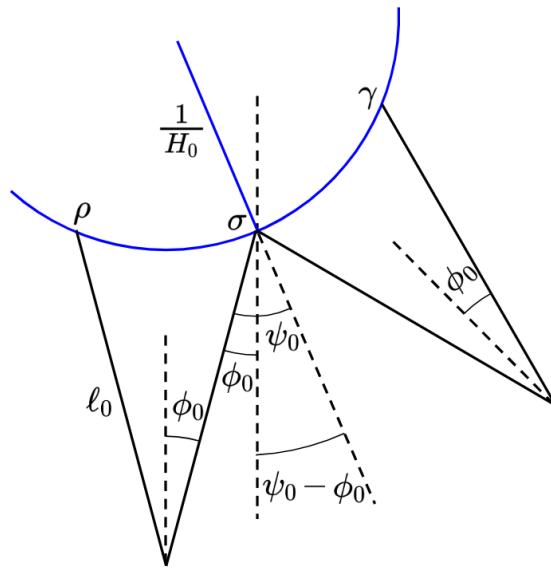


Fig. 2.3 Geometry for relating collar boundary angle ψ to curvature H .

Consider two neighboring cells with collar boundaries a , b , and c , as shown in Figure 2.3. We might imagine defining a radius of curvature by the circle that passes through the three collar boundaries. If we set $\mathbf{x}_a = 2\ell \sin \phi_0 (-1, 0)$, $\mathbf{x}_b = (0, 0)$, and $\mathbf{x}_c = 2\ell \sin \phi_0 (\sin(\psi_0 - \phi_0), \cos(\psi_0 - \phi_0))$, we can solve for the circle coordinates

$$(x_\circ, y_\circ) = \ell \sin \phi_0 \left(-1, \frac{1 + \cos 2(\psi_0 - \phi_0)}{\sin 2(\psi_0 - \phi_0)} \right)$$

to get the inverse radius of curvature

$$H_0 = \frac{1}{\sqrt{x_\circ^2 + y_\circ^2}} = \frac{\sin(\psi_0 - \phi_0)}{\ell \sin \phi_0}. \quad (2.8)$$

This has a nice, simple interpretation in that if $\psi_0 > \phi_0$, $H_0 > 0$ (as drawn in Figure 2.3). On the other hand, $\psi_0 < \phi_0$ implies $H_0 < 0$, or the sheet is concave on the cell body side.

Alternatively, we solve for ψ_0 as a function of H_0 ,

$$\psi_0 = \phi_0 + \arcsin(H_0 \ell \sin \phi_0). \quad (2.9)$$

As we find later in equation 2.17, the curvature of the sheet in any given direction is greater than or equal to $-1/\ell$ (cell bodies and collars cannot go through each other). This lower bound corresponds in 2.9 to $\psi_0 = 0$, which we expect when cells are pressing tightly against each other (Figure 2.5).

2.2.2 Problem statement

Powers [47] shows that for an energy density \mathcal{E} written in terms of the first and second fundamental forms $g_{\alpha\beta}$ and $K_{\alpha\beta}$, we can write an expression for the stress tensor \mathbf{F}^α

$$\mathbf{F}^\alpha = \left(T^{\alpha\beta} + \mathcal{E}^{\alpha\beta} K_\gamma^\beta \right) \mathbf{t}_\beta - (\nabla_\beta \mathcal{E}^{\alpha\beta}) \hat{\mathbf{n}}, \quad (2.10)$$

where summation over repeated indices is implied. Here, $K_\gamma^\beta = K_{\gamma\delta} g^{\delta\beta}$, $\mathbf{t}_\beta = \partial_\beta \mathbf{r}$, and

$$\begin{aligned} T^{\alpha\beta} &= g^{\alpha\beta} \mathcal{E} + 2 \frac{\partial \mathcal{E}}{\partial g_{\alpha\beta}} = \frac{2}{\sqrt{g}} \frac{\partial}{\partial g_{\alpha\beta}} (\sqrt{g} \mathcal{E}) \\ \mathcal{E}^{\alpha\beta} &= \frac{\partial \mathcal{E}}{\partial K_{\alpha\beta}} \end{aligned}$$

with $g = \det g_{\alpha\beta}$.

Since the force \mathbf{f} acting on a surface point is given by the covariant divergence of the stress $\nabla_\alpha \mathbf{F}^\alpha$, our problem is effectively solved once we decide on an appropriate energy density. I discuss two choices: one which introduces the energy density with separate bending and stretching energies from continuum mechanics and another developed by studying the geometry specific to *C. flexa* cells. Brunet et al. [7] showed that the angle formed by the *C. flexa* collars changes when individual cells are triggered for inversion. We might reasonably suggest preferred sheet curvature is prescribed by changing the preferred angle of the collar

ϕ_0 and imposing an energetic cost based on the amount that the collar angle $\phi(\theta)$ differs around the collar in θ : $\mathcal{E} \sim \int (\phi(\theta) - \phi_0)^2 d\theta$.

2.2.3 Continuum surface mechanics approach

We begin by describing the *C. flexa* sheet as a thin plate with large deflections to define an energy density in terms of the strain tensor and displacement. The displacement of a surface defined in the xy -plane can be described with a displacement vector function $\mathbf{u}(\mathbf{r})$ and deflection function $\zeta(\mathbf{r})$ so that $(x, y, 0)$ is mapped to $(x + u_x, y + u_y, \zeta)$ [30]. Using the chain rule, we see that an infinitesimal length squared ds^2 on the surface $dx^2 + dy^2$ becomes

$$\begin{aligned} ds'^2 &= (dx + du_x)^2 + (dy + du_y)^2 + d\zeta^2 \\ &= \left(dx + \frac{\partial u_x}{\partial x} dx \right)^2 + \left(dy + \frac{\partial u_y}{\partial y} dy \right)^2 + \left(\frac{\partial \zeta}{\partial x} + \frac{\partial \zeta}{\partial y} \right)^2 \\ &= \delta_{ij} dx_i dx_j + \left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} + \frac{\partial \zeta}{\partial x_i} \frac{\partial \zeta}{\partial x_j} \right) dx_i dx_j \\ &= (\delta_{ij} + u_{ij}) dx_i dx_j, \end{aligned}$$

where i, j index over 1 and 2. The strain tensor u_{ij} , which describes infinitesimal displacement, is typically used to define a quadratic energy

$$\mathcal{E}_s = \frac{1}{2} \int_S \sum_{i,j} (2\mu u_{ij}^2 + \lambda u_{kk}) d^2 r \quad (2.11)$$

as an infinitesimal form of Hooke's law [30].

The bending energy of membranes is often prescribed to be the Helfrich bending energy [21],

$$\mathcal{E}_b = \int_S \frac{\kappa}{2} (2H - 2H_0)^2 + \kappa_G K d^2 r. \quad (2.12)$$

Here $H = 1/2(g_{\mu\nu}K^{\mu\nu})$ is the mean curvature of the sheet given by \mathbf{u} and ζ and $K = \det K_\mu^\nu$. Since *C. flexa* sheets do not form closed surfaces, any shape equation from equation (2.12) must come with appropriate boundary conditions. Tu and Ou-Yang [58] derived a general equilibrium solution to equation (2.12) with boundary, as well as the simplified axisymmetric case, by taking the functional variation of equation (2.12) with respect to the surface position.

Seung and Nelson [51] derived the equilibrium solution to the simultaneous stretching and bending problem in the limit of small $|\nabla \zeta|$, analogous to the approximation used in

section 2.1. The resulting equations are too difficult to solve [30], but the authors find that Gaussian curvature effectively cancels stress due to disclinations and dislocations, or topological defects in the underlying surface crystal structure [50]. The shape equations derived from functional variations of equations (2.11) and (2.12) may be solved in the inextensional limit that $\mu, \lambda \rightarrow \infty$ and show that topological defects produce buckling.

Since we know well that collars may vary in length, the limit solution to the resulting shape equations is unrealistic and we would resort to treating the problem numerically. Additionally, as in section 2.2.1, sheet bending and collar angle (hence, sheet stretching) are coupled. Due to this coupling, I aimed to write a combined energy density for use in equation (2.10) with the goal of deriving a more manageable solution.

2.2.4 Connecting continuous surface with individual cell mechanics

Following cues from the experimental descriptions of *C. flexa* collars, I sought to write an energy density in terms of the collar angle ϕ . Since ϕ may vary around a cell at some position on the sheet, I write $\phi(\theta)$ as a function of angle θ varying from 0 to 2π around the cell. Then the energy density is written proportionally to the integral of squared deviation of ϕ from a reference collar angle ϕ_0 ,

$$\mathcal{E} = \int_0^{2\pi} (\phi(\theta) - \phi_0)^2 d\theta. \quad (2.13)$$

I proceed by deriving the relationship between $\phi(\theta)$ and the second fundamental form $K_{\mu\nu}$ which describes the sheet curvature.

For any point on a smooth surface, we could find an orthonormal basis of eigenvectors e_1, e_2 in the tangent space that diagonalises K_μ^ν . In terms of a vector $\Delta\xi$ written in this basis, we have that the change in height with respect to the tangent plane and its normal Δh is given by $K_{\mu\nu}\Delta\xi^\mu\Delta\xi^\nu$.

If the cell has an optimal collar angle ϕ_0 and corresponding optimal curvature $K_{0\mu\nu}$, then the height of the collar will be $K_{0\mu\nu}\Delta\xi_0^\mu\Delta\xi_0^\nu$. The distance from the centerline of the cell (the norm of $\Delta\xi_0$) is determined by ϕ_0 . The geometry is shown in Figure 2.4.

If the cell has collar angle ϕ in direction θ , we know the collar distance as $\Delta\xi = \ell(\cos\phi, \sin\phi)$.¹ For a fixed collar length ℓ , we know the difference in height between the ground state and deformed state is $\ell(\cos\phi - \cos\phi_0)$. We can then relate the collar angle and sheet curvature by equating

¹These calculations are temporarily done in the cell-collar plane, assuming that the collar extends only radially from the apicobasal axis. Figure 2.4 is drawn showing geometry in this plane.

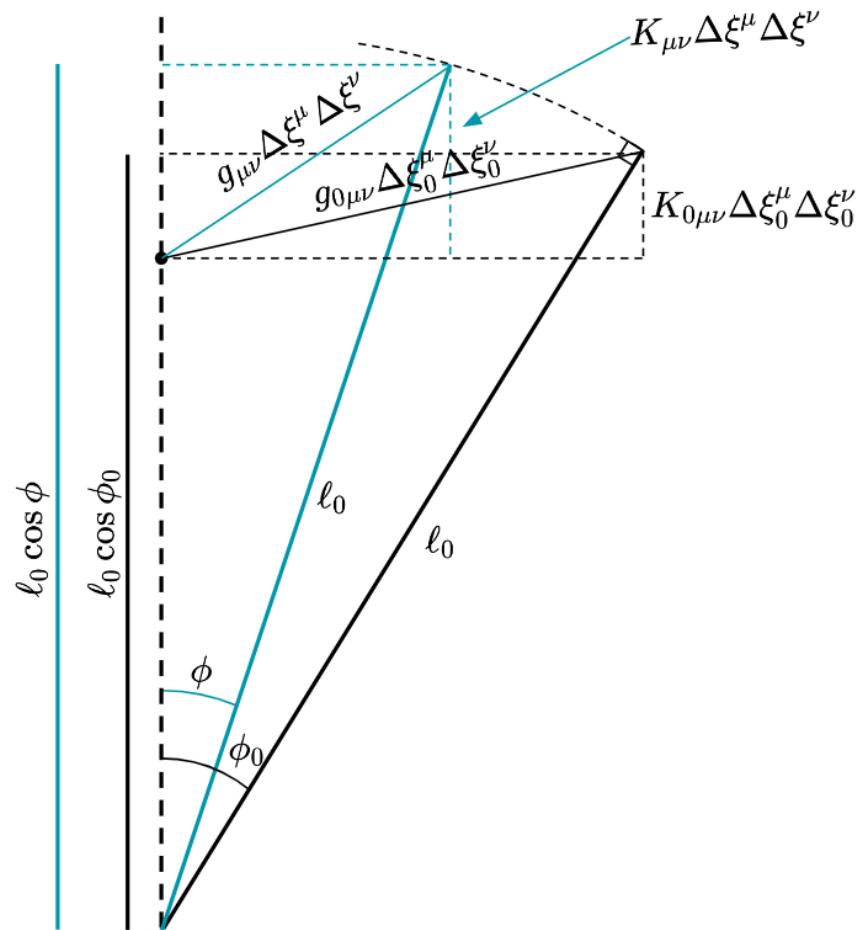


Fig. 2.4 Geometry of a single cell and collar with a continuous surface approximating the interactions between collars.

$$K_{\mu\nu}\Delta\xi^\mu\Delta\xi^\nu - K_{0\mu\nu}\Delta\xi_0^\mu\Delta\xi_0^\nu = \ell(\cos\phi - \cos\phi_0). \quad (2.14)$$

The radius out from the center for the ground state is $\ell \sin\phi_0$ while for the deformed state it is $\ell \sin\phi$, so for $K_{011} = K_{022} = H_0$, we get

$$\begin{aligned} K_{\mu\nu}\ell^2 \sin^2\phi (\cos\theta, \sin\theta)^{\mu,\nu} - H_0\ell^2 \sin^2\phi_0 &= \ell(\cos\phi - \cos\phi_0) \\ H_\theta\ell^2 \sin^2\phi - H_0\ell^2 \sin^2\phi_0 &= \ell(\cos\phi - \cos\phi_0) \end{aligned}$$

where $H_\theta = K_{11}\cos^2\theta + K_{22}\sin^2\theta + 2K_{12}\sin\theta\cos\theta$ is the curvature of a line on the surface in direction θ . We can cancel a factor of ℓ , redefine units of length in terms of ℓ (such that H_θ is the ratio of ℓ with the radius of curvature in direction θ), and express $\sin^2\phi$ in terms of $\cos\phi$ to get

$$\begin{aligned} 0 &= H_\theta \cos^2\phi + \cos\phi + (H_0 \sin^2\phi_0 - \cos\phi_0 - H_\theta) \\ \cos\phi &= \frac{-1 \pm \sqrt{1 + 4H_\theta(H_\theta + \cos\phi_0 - H_0 \sin^2\phi_0)}}{2H_\theta}. \end{aligned}$$

If we take the collars to always have angle $0 \leq \phi \leq \pi/2$, we can constrain $0 \leq \cos\phi \leq 1$ to find the two inequalities

$$H_\theta \geq H_0 \sin^2\phi_0 - \cos\phi_0 \quad (2.15)$$

$$1 \geq \cos\phi_0 - H_0 \sin^2\phi_0. \quad (2.16)$$

The second inequality can be simplified to $H_0 \geq -(1 + \cos\phi_0)^{-1}$. Combining the two inequalities yields

$$H_\theta \geq -1. \quad (2.17)$$

Re-expressed with units, equation (2.17) expresses that $H_\theta \geq -1/\ell$, or that the radius of curvature can never be smaller than ℓ on the cells' side. Physically, we understand this to mean that cells are unable to push through each other (figure 2.5).

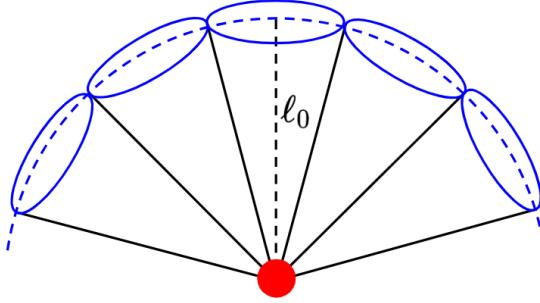


Fig. 2.5 Maximum cell-side curvature is given by inequalities 2.15, 2.16 to have radius ℓ . This corresponds to every (point) cell bumping into each other.

2.2.5 Writing the energy

We want to write equation 2.14 in terms of $\phi - \phi_0$ to write down the energy. We can use a trigonometric identity to write

$$H_\theta \sin^2 \phi - H_0 \sin^2 \phi_0 = -2 \sin \frac{\phi - \phi_0}{2} \sin \frac{\phi + \phi_0}{2}. \quad (2.18)$$

If $\phi - \phi_0$ is small in magnitude, then

$$\begin{aligned} \sin \frac{\phi - \phi_0}{2} &\approx \frac{\phi - \phi_0}{2} \\ \sin \frac{\phi + \phi_0}{2} &= \sin \left(\phi_0 + \frac{\phi + \phi_0}{2} \right) \approx \sin \phi_0 + \frac{\phi - \phi_0}{2} \cos \phi_0 \\ \sin^2 \phi &= \sin^2 (\phi_0 + (\phi - \phi_0)) \approx \sin^2 \phi_0 + (\phi - \phi_0) \sin 2\phi_0. \end{aligned}$$

Using these approximations we rewrite equation 2.18 to first order in $\phi - \phi_0$ as

$$\begin{aligned}
(H_\theta - H_0) \sin^2 \phi_0 + H_\theta (\phi - \phi_0) \sin 2\phi_0 &= -(\phi - \phi_0) \sin \phi_0 \\
(H_\theta - H_0) \sin^2 \phi_0 &= -(\phi - \phi_0)(H_\theta \sin 2\phi_0 + \sin \phi_0) \\
\frac{(H_\theta - H_0)^2 \sin^4 \phi_0}{(H_\theta + \sin \phi_0 / \sin 2\phi_0)^2 \sin^2 2\phi_0} &= (\phi - \phi_0)^2.
\end{aligned}$$

To integrate this function in θ (equation (2.13)), let $K_{11} = a$, $K_{22} = b$, $K_{12} = c$, $-H_0 = d$ and $\sin \phi_0 / \sin 2\phi_0 = e$ for simplicity when we write

$$\int_{-\pi}^{\pi} (\phi - \phi_0)^2 d\theta = \int_{-\pi}^{\pi} \frac{(a \cos^2 \theta + b \sin^2 \theta + 2c \sin \theta \cos \theta + d)^2}{(a \cos^2 \theta + b \sin^2 \theta + 2c \sin \theta \cos \theta + e)^2} d\theta. \quad (2.19)$$

The integral in equation (2.19) can be solved analytically and expressed in terms of H and K (Appendix A),

$$\int_{-\pi}^{\pi} (\phi - \phi_0)^2 d\theta = \text{const.} + \text{const.} \frac{4K + 2(d + 3e)H + 2de + e^2}{(K + 2H + e^2)^{3/2}}. \quad (2.20)$$

Varying the energy density defined by equation (2.20) (from equation (2.13)) is more challenging than the Helfrich membrane energy density (equation (2.12)) since there is no term linear in K . As a result, the Gauss-Bonnet theorem $\int_S K d^2 r = 2\pi n + \oint_{\partial S} K ds$ for an integer n based on surface topology cannot be used to reduce the effects of Gaussian curvature to boundary conditions.

2.2.6 Energy variation

The energy density equation (2.20) can be varied directly or used in equation (2.10) to solve forces on the sheet or the equilibrium shape equations. While the functional variation is possible and not too challenging for the surface interior (Appendix A), the resulting shape equation is clearly unapproachable in a simple way. Since obtaining an approximate solution would require a numerical implementation, we are better suited to treat the sheet as the interactions between several discrete units.

Chapter 3

Discrete model

Models of organisms and population dynamics must be designed by either describing potentially many discrete units¹ with continuous functions or dealing with the analytical complexity of discretisation. Even with the continuous description of *C. flexa* described previously, numerical solutions to the shape equations would require discretisation of a surface. Here, I pursue a simplified, discrete description of *C. flexa* sheets. As in chapter 2, I proceed by formulating an energy function based on the coordinate vectors of cells and vary it with respect to the coordinates. This procedure results in expressions for the forces on the coordinates, which can be forward integrated in time to study the dynamics and steady state geometries of *C. flexa* sheets.

In addition to avoiding the complexity of describing surface curvatures H, K in a discrete program, I find the force equations are relatively quite tractable. Expressing the gradient of the energy as summations over collars belonging to each cell or cell-cell interactions makes it possible to neatly arrange the force calculations as tensor index contractions or, even more simply, matrix multiplications. Of course, describing choanoflagellate sheets as discrete systems also remains faithful to real sheets, and this approach remains applicable over colonies of all cell counts.

3.1 Discrete sheet description

The most natural framework to describe interactions between a set of discrete points is graph theory. Since colonies of *C. flexa* form sheets with two distinct sides and there is no evidence to suggest that *Choanoeca* acts otherwise [7, 35], we are welcome to treat the

¹in this case, cells

network of interactions between cells as a plane graph G . With cells $C = \{\alpha\}_{\alpha \in C}$ making up vertices and cell-cell interactions via collars $E = \{(\alpha, \beta)\}_{(\alpha, \beta) \in E}$ making up edges in a graph G , we describe which cells impart forces on each other.

However, we understand from the studies into *Choanoeca* that cells interact through their collars [14, 35, 7]. Guided by imaging in the sheet-tangential plane (??), we see that another natural planar graph G^* is that with edges along interfaces between cells' collars.² In this description, vertices mark the points at which the interface between a pair of cells $(\alpha, \beta) \in E$ ends. For a cell $\alpha \in C$ whose collar microvilli are all in contact with those of other cells, the vertices in G^* corresponding to cell α are points where α interacts with two or more cells simultaneously.

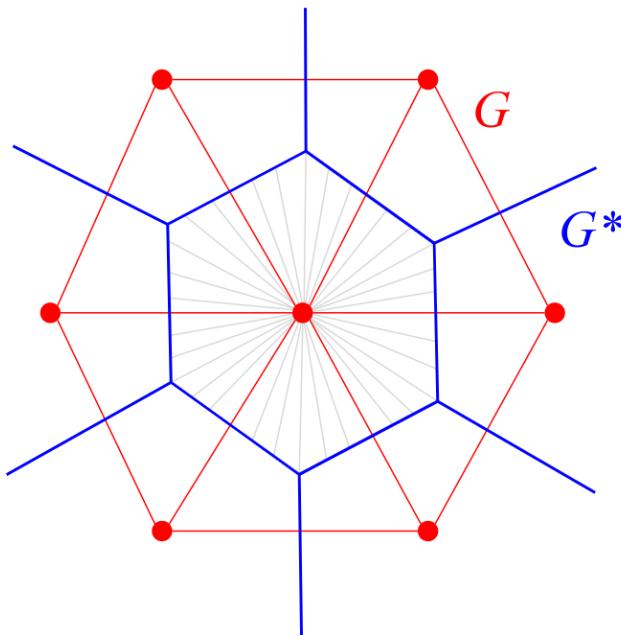


Fig. 3.1 Planar dual graphs describing *C. flexa* colonies. The graph of cells and cell-cell interactions G shown in red and collar interfaces G^* shown in blue.

²Until noted otherwise, I treat the several collars interacting between two cells as a continuous line of interaction with infinitesimal collars spanning the cells' interface. The implications of this choice are discussed later.

As each edge in G^* transects an edge (α, β) between cells α, β in G , we identify G^* as the dual graph of G (figure 3.1).³ Consequently, we have that either G or G^* is sufficient to specify the topology of the other provided the coordinates of vertices.

3.1.1 Defining a sheet

A reasonable simplification of the cell sheet will use either the cell-cell interaction graph G or the collar boundary edge graph G^* . Since physical interactions occur at the collar interfaces, I consider first the physical details of a model defined on G^* .

If two cells have a collar boundary described by $\mathbf{r}_\rho t + (1-t)\mathbf{r}_\sigma$ with $0 \leq t \leq 1$ and the energy is defined by continuously many springs from the boundary to a projected cell point \mathbf{r}_α^* , then the energy $E_{\rho\sigma}$ of that boundary is

$$E_{\rho\sigma} = \int_0^1 [(\mathbf{r}_\rho t + (1-t)\mathbf{r}_\sigma) - \mathbf{r}_\alpha^*]^2 dt = \frac{1}{3}(\mathbf{r}_\rho + \mathbf{r}_\sigma)^2 - \frac{1}{3}\mathbf{r}_\rho \cdot \mathbf{r}_\sigma - \mathbf{r}_\alpha^* \cdot (\mathbf{r}_\sigma + \mathbf{r}_\sigma) + \mathbf{r}_\alpha^{*2}. \quad (3.1)$$

The energy corresponding to a cell consists of the line energies of all the collar interfaces. We find the position \mathbf{r}_α^* by setting the gradient of equation 3.1 with respect to \mathbf{r}_α^* to zero for all lines $\rho\sigma$. If ρ indexes the vertices that cell α has (which I denote $\rho \in \alpha$ for the remainder of this chapter), then

$$\begin{aligned} 0 &= \frac{dE}{d\mathbf{r}_\alpha^*} = - \sum_{\rho \in \alpha} \mathbf{r}_\sigma + 2d_\alpha \mathbf{r}_\alpha^* \\ \mathbf{r}_\alpha^* &= \frac{1}{d_\alpha} \sum_{\rho \in \alpha} \mathbf{r}_\rho \end{aligned}$$

for total stretching energy $E = \sum_{\rho\sigma} E_{\rho\sigma}$ and number of collars d_α belonging to α .

The force on vertex σ is then given by the negative gradient of the whole sheet energy E , which is the sum of the energies corresponding to each cell α :

$$\frac{dE}{d\mathbf{r}_\sigma} = \frac{d}{d\mathbf{r}_\sigma} = \sum_{\alpha: \sigma \in \alpha} \frac{d}{d\mathbf{r}_\sigma} \sum_{\rho \in \alpha} (\mathbf{r}_\rho - \mathbf{r}_\alpha^*)^2.$$

³As G is finite, all vertices in G^* as described above which indicate two cells' interactions ending at the edge of a colony sheet would be merged into a single vertex v_* in the dual graph of G . Consequently G^* is not exactly the dual graph of G . We address this later when defining sheet edges numerically by replacing each edge (v, v^*) in G^* with the edge (v, v') for new vertices v' and removing v_* from G^* and deleting all edges incident on v_* .

Since \mathbf{r}_α^* depends on \mathbf{r}_ρ itself, we write

$$\begin{aligned} -\frac{dE}{d\mathbf{r}_\sigma} &= \sum_{\alpha:\sigma \in \alpha} \frac{d}{d\mathbf{r}_\sigma} \sum_{\rho \in \alpha} \left(\mathbf{r}_\rho - \frac{1}{d_\alpha} \sum_{\gamma \in \alpha} \mathbf{r}_\gamma \right)^2 \\ &= \sum_{\alpha:\sigma \in \alpha} \sum_{\rho \in \alpha} \left(2\mathbf{r}_\sigma \delta_{\sigma\rho} - \frac{2}{d_\alpha} \sum_{\gamma \in \alpha} (\mathbf{r}_\gamma \delta_{\sigma\rho} + \mathbf{r}_\rho \delta_{\sigma\gamma}) + \frac{1}{d_\alpha^2} \sum_{v \in \alpha} \sum_{v \in \alpha} (\mathbf{r}_v \delta_{\sigma v} + \mathbf{r}_v \delta_{\sigma v}) \right) \\ &= -2 \sum_{\alpha:\sigma \in \alpha} (\mathbf{r}_\sigma - \mathbf{r}_\alpha^*). \end{aligned} \quad (3.2)$$

Equation (3.2) aligns with the expectation that the forces on \mathbf{r}_σ are linear based on the difference to the projected cell points \mathbf{r}_α^* that σ belongs to, owing to the linearity of the entire system. While the answer may have been readily guessed, the above process of solving for the forces on \mathbf{r}_σ are necessary for nonzero collar length ℓ_0 .

If instead the line energy is

$$E_{\rho\sigma} = \int_0^1 (|\mathbf{r}_\rho t + (1-t)\mathbf{r}_\sigma - \mathbf{r}_\alpha^*| - \ell_0)^2 dt,$$

then the cell position \mathbf{r}_α^* is the solution to

$$0 = -2 \sum_{\rho \in \alpha} \mathbf{r}_\rho + 2d_\alpha \mathbf{r}_\alpha^* - 2\ell_0 \frac{d}{d\mathbf{r}_\alpha^*} \sum_{(\rho,\sigma) \text{ edge in } \alpha} \int_0^1 \frac{\mathbf{r}_\alpha^* - \mathbf{r}_\sigma - t(\mathbf{r}_\rho - \mathbf{r}_\sigma)}{|\mathbf{r}_\rho t - (1-t)\mathbf{r}_\sigma - \mathbf{r}_\alpha^*|} dt \quad (3.3)$$

Although the integral in equation (3.3) can be evaluated analytically, it results in a transcendental equation for \mathbf{r}_α^* . Despite the complexity of nonzero equilibrium length for finding a cell position, I nevertheless use the result that continuous spring interfaces can be effectively distilled to interface endpoints (equation (3.2)) in the model developed in section 3.1.3.

Bending

Points corresponding to cell bodies and collar-collar interface edges make it simple to describe a physically realistic bending energy, though it is not as clear how to define a bending energy.

Seung and Nelson [51] describe triangulated surfaces and define a bending energy between two triangles α, β sharing an interface ρ, σ as contributing bending energy $|\hat{\mathbf{n}}_\alpha - \hat{\mathbf{n}}_\beta|^2/2 = 1 - \hat{\mathbf{n}}_\alpha \cdot \hat{\mathbf{n}}_\beta$, where all normals face on the same side of the surface. Indeed, the continuous approximation of this energy function for small difference $\hat{\mathbf{n}}_\alpha - \hat{\mathbf{n}}_\beta$ gives the

Helfrich energy density $4H^2 - 2K$ [21]. When cells make boundaries with more than three cells simultaneously, there is not an immediately clear way to define the normal vector $\hat{\mathbf{n}}_\alpha$ of a cell α .

As I discuss later (section 3.2.1), a reasonable choice that does not depend on cell position (which in this case is already projected onto the approximated plane of collar positions) would be to approximate $\hat{\mathbf{n}}_\alpha$ as the normal vector of a plane approximation to the collar vertices \mathbf{r}_ρ for $\rho \in \alpha$. Although this choice is intuitively reasonable, it lacks physical motivation as it does not necessarily minimise the energy when the cell body is considered (section 3.2.1). On the other hand, treating the normal vectors as free variables here would allow them to take any direction to minimise the energy, which does not translate to any physical movement or rotation of the cell body.

3.1.2 Surface formed by cell bodies

The other natural, minimal discrete description of *C. flexa* is to include only cell coordinates and the network of cell-cell interactions (section 3.1). Again, we look to describe the energy contributed by collar stretching and sheet bending. Unlike the description of bending in section 3.1.1, knowledge of cell body positions gives cell orientation vectors $\hat{\mathbf{n}}_\alpha$ a clear physical significance. Defining the $\hat{\mathbf{n}}_\alpha$ as free vectors removes any ambiguity and a bending energy $1 - \hat{\mathbf{n}}_\alpha \cdot \hat{\mathbf{n}}_\beta$ for two cells α, β is reasonable.

On the other hand, considering only cell positions gives no clear method for defining a stretching energy based in a physical mechanism. We may proceed to define both bending and stretching energy by solving for the minimum energy curves describing two filaments meeting at some mutual point and tangent angle as functions of cell body orientation vectors $\hat{\mathbf{n}}_\alpha, \hat{\mathbf{n}}_\beta$ and vector $\mathbf{r}_\alpha - \mathbf{r}_\beta$ between the two cells. For any description of cell-cell interactions in *C. flexa* sheet, this approach by treating each collar as a linear filament would likely be the most accurate as it does not require any simplification into angles ϕ, ψ (equations (3.7) and (3.11)). However, this level of complexity removes the interpretational and computational simplicity of distilling the problem as I sought. Since neither approach here nor in section 3.1.1 captures all physical interactions in a *C. flexa* sheet with clearly defined terms, I decided to pursue an approach that used details of both collar interactions and cell positions.

3.1.3 Numerically specifying initial conditions

For sheets whose graph of cell-cell connections G can be drawn on a plane with edges as straight lines, it is simplest to define the initial spatial graph G as lying in the xy -plane with

cell coordinates $\{\mathbf{r}_\alpha\}_{\alpha \in C}$ and to use Voronoi tessellation to generate collar vertices in G^* . Voronoi tessellation allows for generalisation beyond regular lattices, though it does not facilitate graph topologies that cannot be drawn in a plane as described above.

To build more complex graphs G that are nevertheless planar, we can either use subsets of common polyhedra (e.g. subdivided icosahedron) or triangulate points that lie along a surface. In the latter case, I found sufficient flexibility in randomly sampling a specified number of points uniformly distributed on a sphere below some latitude, then calculating the generalised Voronoi tessellation with respect to the metric on the sphere as described in Caroli et al. [8].

We build introduce vertices from G^* where two cells' collar microvilli diverge to build a larger graph \mathfrak{G} consisting of cell-cell interaction edges (α, β) from G and cell-collar edges (α, ρ) between cells α and collar edges $\rho \in \alpha$. Here, $\rho \in \alpha$ denotes a collar vertex ρ from G^* connecting to cell α via the cell's microvilli. With cell positions \mathbf{r}_α already specified and cell-cell interactions given from G , collar vertex positions \mathbf{r}_ρ of collar vertices connecting to three cells are initially set as the centroid of the three cells plus the normal vector of the triangle they form. The orientation of the normal vector is set to be consistent across the sheet, such that all collar vertices are positioned on the same side of the sheet, as all cells in *C. flexa* colonies face the same direction.⁴

As Voronoi diagrams for finitely many cells include ridges that extend out to infinity, and the spherical Voronoi algorithm on points below a given latitude on the sphere produces ridge vertices above that latitude, collar vertices at the boundary of the sheet must be added manually. I call these collar vertices at the edges as *boundary collars*. Before choosing to add these vertices, we must first consider how collar interactions at the boundary affect sheet energy.

Boundary collar vertices

We ask whether boundary collar vertices contribute to the energy. For boundary cells α, β , suppose the line of physical interactions between the two cells' microvilli spans from point \mathbf{r}_ρ and ends at point \mathbf{r}_σ . We wish to know how the angle between the planes given by points ρ, α, σ and ρ, β, σ changes with the position of boundary collar vertex σ .

Suppose for the time being that the collar length is fixed at ℓ , so the possible values for \mathbf{r}_σ are constrained. We simplify the problem by reparameterising our coordinates such that $\mathbf{r}_\alpha = (-1, 0, 0)$, $\mathbf{r}_\rho = (0, r, 0)$, and $\mathbf{r}_\beta = (1, 0, 0)$, where $r = \sqrt{\ell^2 - 1}$. Here, ℓ is a

⁴Since I use Voronoi triangulation to collar vertices, at most three cells interact at any collar vertex. This is a consequence of the dual of Voronoi tessellation being Delaunay triangulation.

dimensionless ratio of the collar length to half the cell-cell distance. We readily see that $\mathbf{r}_\sigma = (x, y, z)$ is constrained to take values in the circle defined by $r^2 = y^2 + z^2, x = 0$.

Parameterising the positions of $\mathbf{r}_\sigma(\theta) = (0, r\cos\theta, r\sin\theta)$ by angle θ with the second axis, we find the normals for planes ρ, α, σ and ρ, β, σ as

$$\begin{aligned}\hat{\mathbf{n}}_{\rho\alpha\sigma} &= (\mathbf{r}_\sigma - \mathbf{r}_\alpha) \times (\mathbf{r}_\rho - \mathbf{r}_\alpha) \\ &= \frac{(-r^2 \sin \theta, r \sin \theta, r - r \cos \theta)}{r^4 \sin^2 \theta + 2r^2(1 - \cos \theta)} \\ \hat{\mathbf{n}}_{\rho\beta\sigma} &= (\mathbf{r}_\rho - \mathbf{r}_\beta) \times (\mathbf{r}_\sigma - \mathbf{r}_\beta) \\ &= \frac{(r^2 \sin \theta, r \sin \theta, r \cos \theta - r)}{r^4 \sin^2 \theta + 2r^2(1 - \cos \theta)}.\end{aligned}$$

After simplifying, the angle between these two normal vectors is

$$\hat{\mathbf{n}}_{\rho\alpha\sigma} \cdot \hat{\mathbf{n}}_{\rho\beta\sigma} = 1 - \frac{2}{1 + \frac{1}{2r^2} (1 + \tan^2 \frac{\theta}{2})}. \quad (3.4)$$

It is clear that the position of the boundary collar interaction at \mathbf{r}_σ changes the angle between these two planes, given by the arccosine of equation (3.4). In the simplified sheet structure defined in the combined cell-collar graph \mathfrak{G} , this results in a change in energy based on the position of \mathbf{r}_σ . Consequently, as the notation indicates, boundary collar vertices are introduced to \mathfrak{G} connecting between each pair of boundary cells.

Adding boundary collar vertices

Defining initial positions for boundary collar vertices becomes challenging when sheets are not planar, though a reasonable placement is sufficient since the positions will be changed later. For sheets in the xy -plane generated by 2-dimensional Voronoi tesselation (as in figure 3.1), ridges extending out to infinity are removed and replaced with collar vertices at finite distance. The position of a boundary collar vertex σ between cells α, β is calculated as follows. First, a unit vector $\hat{\mathbf{n}}$ perpendicular to the ridge between cell positions $\mathbf{r}_\alpha, \mathbf{r}_\beta$ is determined. For sheets in the xy -plane, $\hat{\mathbf{n}} = \hat{z}$. Otherwise, $\hat{\mathbf{n}}$ is simply aligned with the average of the normal vectors $\hat{\mathbf{n}}_\alpha, \hat{\mathbf{n}}_\beta$. Then, the boundary collar vertex position \mathbf{r}_σ is positioned at the reflection of \mathbf{r}_ρ over the plane given by points $\mathbf{r}_\alpha, \mathbf{r}_\beta, \mathbf{r}_\alpha + \hat{\mathbf{n}}$, where ρ is the existing collar vertex shared by α and β . Notably, this process results in a boundary collar position \mathbf{r}_σ farther from the center of the sheet than \mathbf{r}_ρ and equidistant to $\mathbf{r}_\alpha, \mathbf{r}_\beta$ as \mathbf{r}_ρ .

This process produced reasonable boundary collar vertex positions to initialise sheet dynamics simulations (figure 3.2). For initial collar positions not too far from cells, collars would not overlap or cross over each other, even for collars defined with non-coplanar cell positions.

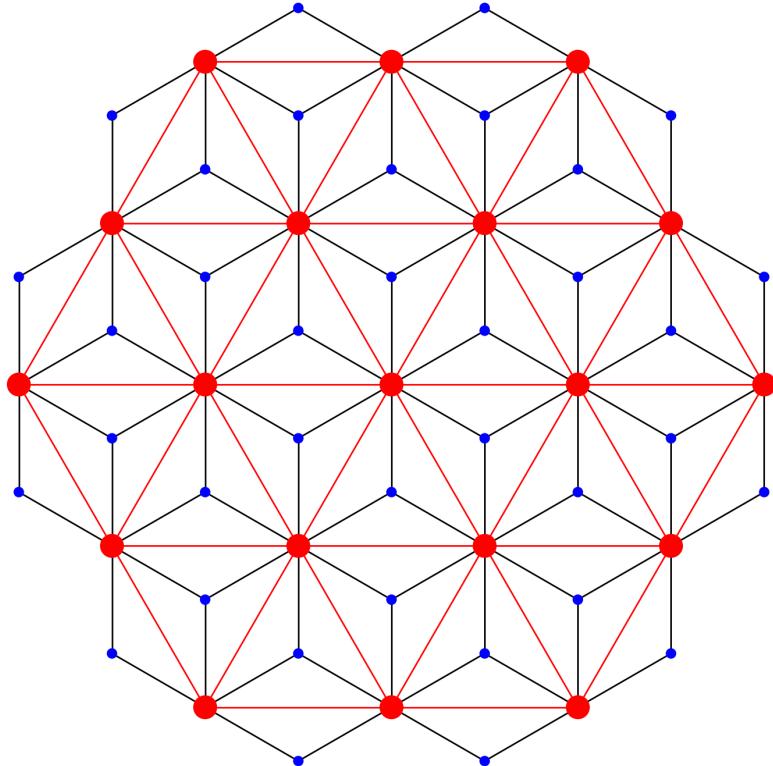


Fig. 3.2 Initial layout for the flexa sheet. Cell bodies are shown in large purple points and collar boundary vertices are shown in small yellow points. Black edges connect cells to collar boundary vertices, and orange edges show cell-cell neighbor relations (though these orange edges are not physically present). The physical interactions are mediated through the black edges.

3.2 Sheet energy

In developing the simplified, discrete model for *C. flexa* as a spatial graph, I aim to distill the complex physics of collar-collar interactions into a minimum number of sufficient energy terms to capture the sheet bending that we observe experimentally. In what follows, I treat

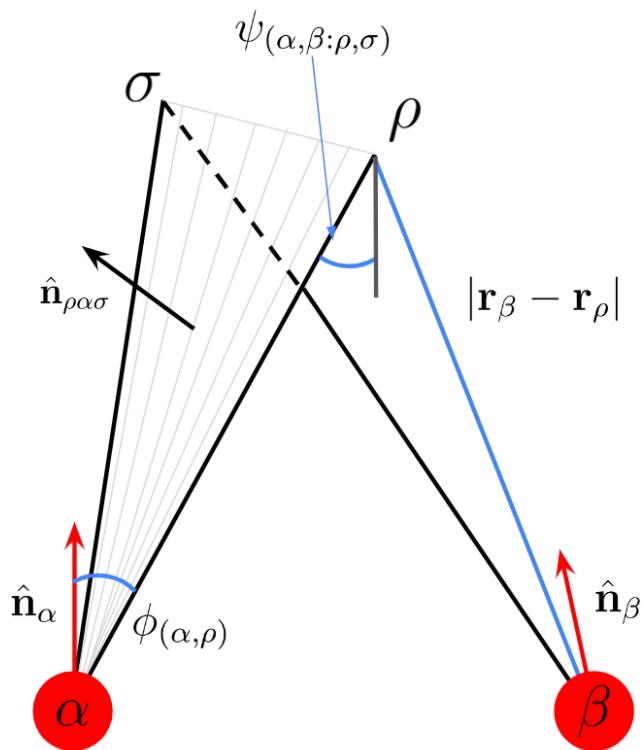


Fig. 3.3 Detailed view of the discrete model for *C. flexa* sheets shown by the interface between two cells, α, β (red), at points ρ, σ . Cells have unit apicobasal axis vectors $\hat{\mathbf{n}}_\alpha, \hat{\mathbf{n}}_\beta$, and the plane containing a cell's position \mathbf{r}_α and two coordinates $\mathbf{r}_\rho, \mathbf{r}_\sigma$ belonging to an interface with another cell is defined $\hat{\mathbf{n}}_{\rho\alpha\sigma}$. Thin grey microvilli are shown for clarity but not included in the model. The energy (equation (3.5)) is defined in terms of angles $\phi_{(\alpha,\rho)}$ and $\psi_{(\alpha,\beta:\rho,\sigma)}$.

edges (α, ρ) between a cell α and collar vertex ρ as straight line collar microvilli and cell pairs, flanking collar pairs $(\alpha, \beta : \rho, \sigma)$ as lines of interactions between the planes given by points ρ, α, σ and ρ, β, σ (figure 3.3).

Consequently, as detailed in the continuous model description of chapter 2, I build an energy function \mathcal{E} that penalises deviations for angles ϕ and ψ , which describe angles between (ϕ) collar microvilli and cell normal vectors $\hat{\mathbf{n}}_\alpha$ and (ψ) plane normals $\hat{\mathbf{n}}_{\rho\alpha\sigma}, \hat{\mathbf{n}}_{\rho\beta\sigma}$:

$$\mathcal{E} = \mathcal{E}_\phi + \mathcal{E}_\psi + \mathcal{E}_{\text{sp}} \quad (3.5)$$

3.2.1 Cell-collar angle energy

For a physical *C. flexa* cell α with fixed position \mathbf{r}_α and fixed collar positions $\{\mathbf{r}_\rho\}_{\rho \in \alpha}$, we realise that the cell still has freedom in its rotation which we expect will contribute substantially to its energy. In other words, there should be an optimal rotation for the cell to minimise its mechanical energy. Since we treat *C. flexa* cells as rotationally symmetric above the apicobasal axis, it suffices in our description to assign to each cell α in the graph \mathfrak{G} a unit vector $\hat{\mathbf{n}}_\alpha$

For simplicity, each vector $\hat{\mathbf{n}}_\alpha$ is initially defined as the unit vector in the direction of $\sum_{\rho \in \alpha} \vec{\alpha\rho}$, where $\vec{\alpha\rho} = \mathbf{r}_\rho - \mathbf{r}_\alpha$.

Defining an energy term on the angle ϕ between a cell α 's collars and the apicobasal axis $\hat{\mathbf{n}}_\alpha$ is established on descriptions of *Choanoeca* in the literature. Brunet et al. [7] describes the change in this angle as the result of exposure to light in *C. flexa*. Similarly, Ellis [14] characterises the variation in ϕ observed in individual cells of *C. perplexa*.

Consequently, we consider an energy term $\mathcal{E}_\phi(\{\mathbf{r}_\alpha, \hat{\mathbf{n}}_\alpha\}, \{\mathbf{r}_\rho\})$ which penalises deviation from a common equilibrium basal collar angle ϕ_0 :

$$\mathcal{E}_\phi(\{\mathbf{r}_\alpha, \hat{\mathbf{n}}_\alpha\}, \{\mathbf{r}_\rho\}) = \sum_{(\alpha, \rho)} (\phi_{(\alpha, \rho)} - \phi_0)^2. \quad (3.6)$$

The sum indicates summation over all cell-collar pairs (α, ρ) in the sheet \mathfrak{G} . The angle $\phi_{(\alpha, \rho)}$ is calculated entirely based on the cell normal vector $\hat{\mathbf{n}}_\alpha$ and unit vector $\hat{\alpha\rho}$ pointing in the direction of $\hat{\mathbf{r}}_\rho - \hat{\mathbf{r}}_\alpha$:

$$\phi_{(\alpha, \rho)} = \arccos(\hat{\mathbf{n}}_\alpha \cdot \hat{\alpha\rho}) = \arccos\left(\hat{\mathbf{n}}_\alpha \cdot \frac{\hat{\alpha\rho}}{|\hat{\alpha\rho}|}\right). \quad (3.7)$$

Optimal cell normal vectors

No other energy terms will depend on the cell normal vectors $\{\hat{\mathbf{n}}_\alpha\}$, so we ask now what the optimal normal vector for a cell is. Suppose a cell is at position \mathbf{r}_α with collar vertices at \mathbf{r}_ρ for $\rho \in \alpha$. We can determine how the cell orients in order to minimise the collar energy with respect to $\hat{\mathbf{n}}_\alpha$.

For fixed α , the cell orientation vector $\hat{\mathbf{n}}_\alpha$ is constrained to have unit length. Hence, we solve the constrained optimisation of \mathcal{E}_ϕ by solving the Lagrange multiplier problem with multiplier λ

$$0 = \frac{\partial [\mathcal{E}_\phi \lambda (|\hat{\mathbf{n}}_\alpha|^2 - 1)]}{\partial \hat{\mathbf{n}}_\alpha} \quad (3.8)$$

$$0 = \frac{\partial [\mathcal{E}_\phi + \lambda (|\hat{\mathbf{n}}_\alpha|^2 - 1)]}{\partial \lambda}. \quad (3.9)$$

Using the constraint (solution to equation (3.9)) $|\hat{\mathbf{n}}_\alpha|^2 = 1$, we solve

$$\begin{aligned} \lambda \hat{\mathbf{n}}_\alpha &= 2 \sum_{\rho \in \alpha} [\arccos(\hat{\alpha}\rho \cdot \hat{\mathbf{n}}_\alpha) - \phi_0] \frac{-1}{\sqrt{1 - (\hat{\alpha}\rho \cdot \hat{\mathbf{n}}_\alpha)^2}} \hat{\alpha}\rho \\ \lambda &= 2 \left| \sum_{\rho \in \alpha} [\arccos(\hat{\alpha}\rho \cdot \hat{\mathbf{n}}_\alpha) - \phi_0] \frac{1}{\sqrt{1 - (\hat{\alpha}\rho \cdot \hat{\mathbf{n}}_\alpha)^2}} \hat{\alpha}\rho \right|. \end{aligned}$$

Then the optimal normal vector solves the transcendental equation

$$\hat{\mathbf{n}}_\alpha = \frac{\sum_{\rho \in \alpha} [\arccos(\hat{\alpha}\rho \cdot \hat{\mathbf{n}}_\alpha) - \phi_0] \frac{-1}{\sqrt{1 - (\hat{\alpha}\rho \cdot \hat{\mathbf{n}}_\alpha)^2}} \hat{\alpha}\rho}{\sum_{\rho \in \alpha} [\arccos(\hat{\alpha}\rho \cdot \hat{\mathbf{n}}_\alpha) - \phi_0] \frac{1}{\sqrt{1 - (\hat{\alpha}\rho \cdot \hat{\mathbf{n}}_\alpha)^2}} \hat{\alpha}\rho} \quad (3.10)$$

Clearly the cell normal vectors must be computed numerically whenever a cell is interacting with several other cells simultaneously in complicated geometries. We can choose to either approximate the normal vectors with a physically reasonable approximation or treat the normal vectors as free arguments to the energy function to be optimised. I discuss both options below.

Approximating cell normal vectors

There are several options for approximating a cell normal vector $\hat{\mathbf{n}}_\alpha$ based on positions \mathbf{r}_α and $\{\mathbf{r}_\rho\}_{\rho \in \alpha}$. The simplest option we might be able to think of is to let $\hat{\mathbf{n}}_\alpha$ be the unit

vector in the direction $\sum_{\rho \in \alpha} \hat{\alpha\rho}$. This approach has the benefit that collar vertices farther from \mathbf{r}_α are weighted more in the cell normal vector, agreeing with the intuition that a more distant collar interaction demands more cell rotation to accommodate it. However, we find that this approach results in unreasonable cell normal vectors for boundary cells as defined in \mathfrak{G} since boundary cells do not have a full ring of boundary collar vertices. For this approach to work, \mathfrak{G} would need to include several more collar vertices which do not describe interactions between cells and add unnecessary complexity. In application, I found that the boundary cell effect of this choice of $\hat{\mathbf{n}}_\alpha(\mathbf{r}_\alpha, \{\mathbf{r}_\rho\}_{\rho \in \alpha})$ substantially affected boundary collar vertex positions after energy equilibration and the overall energy landscape as a function of equilibrium angles ϕ_0, ψ_0 .

When initialising a flat sheet, the above averaging approach also does not agree with intuition, since it results in boundary cell normal vectors that do not point in the same direction as normal vectors for cells not on the sheet boundary (figure 3.2). Instead, when a sheet lies flat in the xy -plane, it is expected that all vectors $\hat{\mathbf{n}}_\alpha$ point in the $+\hat{z}$ direction provided that collars are above cells in \mathbf{z} . A viable alternative is to define $\hat{\mathbf{n}}_\alpha$ by taking a plane approximation to cell α 's collar vertices. The normal vector to this plane oriented away from the cell defines a normal vector that agrees with intuition and supports calculating normals for a non-coplanar set of collar vertices.

The plane approximation approach is easiest achieved using ordinary least squares. Briefly, we approximate $\hat{r}_{\rho 3} = (\mathbf{r}_{\rho 1}, \mathbf{r}_{\rho 2}) \cdot (\beta_1, \beta_2) + \beta_0$ and minimise the sum of squared residuals $\sum_{\rho \in \alpha} (\hat{r}_{\rho 3} - r_{\rho 3})^2$ with respect to $\beta_0, \beta_1, \beta_2$. The normal vector of the plane approximation is then $(\beta_1, \beta_2, -1)$ up to normalisation and multiplication by -1 .⁵

3.2.2 Collar-collar interface angle energy

As in chapter 2, we aim to produce sheet curvature with the angle ψ that two cells' collars make at their interface. As in section 3.1.3, we calculate the angle between planes defined by two cells α, β and their mutual flanking collar vertices ρ, σ with

$$\Psi_{(\alpha, \beta; \rho, \sigma)} = \frac{\pi}{2} - \frac{1}{2} \arccos (\hat{\mathbf{n}}_{\rho \alpha \sigma} \cdot \hat{\mathbf{n}}_{\rho \beta \sigma}). \quad (3.11)$$

The normal vectors $\hat{\mathbf{n}}_{\rho \alpha \sigma}$ for a plane given by points ρ, α, σ must have a systematically defined orientation, as they can point in either direction $\pm(\mathbf{r}_\rho - \mathbf{r}_\alpha) \times (\mathbf{r}_\sigma - \mathbf{r}_\alpha)$. In the geometry used to define ψ in equation (3.11), the collar-cell-collar normal vectors are

⁵The notation here is chosen to be consistent with that typically used in ordinary least squares, hence the hatted values indicate an approximation rather than vector normalisation as I use otherwise.

assumed to be pointing in the direction of the cells' flagella. With the simplifying assumption that the cell normal always points in the inside of the collar, we have that the collar-cell-collar normals must take orientation to align with their corresponding cell normals. Consequently, we let $\hat{\mathbf{n}}'_{\rho\alpha\sigma} = \vec{\alpha}\rho \times \vec{\alpha}\sigma$ and set $\hat{\mathbf{n}}_{\rho\alpha\sigma} = \text{sgn}(\hat{\mathbf{n}}'_{\rho\alpha\sigma} \cdot \hat{\mathbf{n}}_\alpha) \hat{\mathbf{n}}'_{\rho\alpha\sigma}$. Here $\text{sgn} : \mathbb{R} \rightarrow \mathbb{R}$ is the sign operator: $\text{sgn}(x) = 1$ if $x \geq 0$ and $\text{sgn}(x) = -1$ if $x < 0$.

Defining $\hat{\mathbf{n}}_{\rho\alpha\sigma}$ in this way makes it dependent on the cell normal vectors $\hat{\mathbf{n}}_\alpha$. However, when minimising the energy, I work to develop methods such that $\text{sgn}(\hat{\mathbf{n}}_{\rho\alpha\sigma} \cdot \hat{\mathbf{n}}_\alpha)$ remains constant. This corresponds to ensuring that no collar vertices cross through each other (corresponding to microvilli crossing over each other) and that the cell normal vectors remain pointing inside the collars. Hence, I effectively treat the effect of $\hat{\mathbf{n}}_\alpha$ on a collar-cell-collar normal vector $\hat{\mathbf{n}}_{\rho\alpha\sigma}$ to be constant.

With a common equilibrium collar-collar interface angle ψ_0 (as all cells are assumed equal in their mechanical properties), we express the energy \mathcal{E}_ψ

$$\mathcal{E}_\psi = k_\psi \sum_{(\alpha,\beta:\rho,\sigma)} (\psi_{(\alpha,\beta:\rho,\sigma)} - \psi_0)^2. \quad (3.12)$$

3.2.3 Collar length

To provide sufficient flexibility to sheets through collar microvilli, I introduce an energy term \mathcal{E}_{sp} defined by

$$\mathcal{E}_{sp} = k_{sp} \sum_{(\alpha,\rho)} |\vec{\alpha}\rho - \ell_{0\alpha\rho}|^2, \quad (3.13)$$

where $\ell_{0\alpha\rho}$ is an equilibrium length for edge (α,ρ) . The sum indicates summation over all cell, collar pairs (α,ρ) in the sheet \mathfrak{G} .

All cells are assumed to take identical properties, so all values $\ell_{0\alpha\rho}$ are set to a constant ℓ_0 unless indicated otherwise. When done so, we find that ϕ_0 , ψ_0 , and ℓ_0 allow us to calculate a natural length scale for the problem, $1/H_0$ (equation (2.8)).

When interested in sheets with constrained collar length, we may either take a numerical approach or exploit the above energy term by setting k_{sp} to a large value. The former is discussed in ??.

3.3 Minimising sheet energy

The sheet energy $\mathcal{E}(\{\mathbf{r}_\gamma\}_{\gamma \in \mathfrak{G}}$ (equation (3.5)) is parameterised over the cell and collar boundary vertex coordinates given a function $\hat{\mathbf{n}}_\alpha(\mathbf{r}_\alpha, \{\mathbf{r}_\rho\}_{\rho \in \alpha})$ that approximates the cell normal for all cells α . When sheet topology is left fixed, the summations in equations (3.6), (3.12) and (3.13) remain unchanged.

I began by using a numerical optimisation routine to minimise \mathcal{E} with respect to the cell coordinates for sheets generated as in section 3.1.3. Sheets with a regular hexagonal lattice permit sheet bending for sufficiently few cells at the sheet boundary. For sheets which are too large, buckling at the sheet boundary emerges due to the inability for collars to compress enough to accommodate the preferred curvature throughout the rest of the sheet.

3.3.1 Numerical optimisation

I minimised energy using the sequential quadratic programming algorithm implemented in the Python module `scipy` [29]. All code written and used in this chapter is available online at <https://github.com/adkonk/flexa>.

Constant collar length constraint

I began with sheets at fixed collar length to study flexibility only at the joints with angles ϕ and ψ . For sheets beginning with a regular hexagonal lattice, we are able to fix all collar lengths to ℓ_0 , the common collar length at the initial condition. The use of a numerical optimisation algorithm makes it straightforward to introduce constraints. Minimising $\mathcal{E} = \mathcal{E}_\phi + \mathcal{E}_\psi + \mathcal{E}_{sp}$ (equation (3.5)) is equivalent to minimising $\mathcal{E}_\phi + \mathcal{E}_\psi$ alone in this context since \mathcal{E}_{sp} is fixed.

Flat sheet as a solution

A flat sheet generated with a hexagonal lattice has a regular structure that makes $\phi_{\alpha\rho} = \phi_0$, $\psi_{(\alpha,\beta:\rho\sigma)} = \psi_0$, $|\mathbf{r}_\alpha - \mathbf{r}_\rho| = \ell_0$ for all cell-collar pairs (α, ρ) and collar-collar interfaces $(\alpha, \beta : \rho, \sigma)$. Consequently, all terms in \mathcal{E} (equation (3.5)) are zero. Since \mathcal{E}_ϕ and \mathcal{E}_ψ are nonnegative, the flat sheet is a stable solution for appropriate ϕ_0, ψ_0 .

Moreover, given equation (2.8) describing sheet curvature in terms of ϕ_0, ψ_0 and the geometry of collar interactions (figure 2.3), we expect that any pair (ϕ_0, ψ_0) where $\phi_0 = \psi_0$ will give a zero energy, flat sheet. A landscape of minimum sheet energies over several pairs (ϕ_0, ψ_0) is expected to a minimum energy valley along the diagonal.

3.3.2 Energy gradient descent

Due to the instability of the numerical optimisation routine used above, I moved to minimising the total energy \mathcal{E} explicitly using gradient descent. The goal in taking this approach is to explicitly calculate the gradients $\partial\mathcal{E}/\partial\mathbf{r}_\gamma$ of the energy with respect to all coordinate vectors \mathbf{r}_γ and incrementally take small steps in the reverse direction.

Although gradient descent in several contexts is criticised for being slow and by nature prone to be trapped in local minima, in the context of modeling *C. flexa* sheets it is preferable to a black box optimisation routine. Calculating the gradient analytically amounts to calculating the forces on all coordinates, and taking incremental steps in the direction of the negative gradient amounts to forward integrating the force $F_\gamma = -\partial\mathcal{E}/\partial\mathbf{r}_\gamma$. As in chapter 2, we assume linear drag such that $\zeta d\mathbf{r}_\gamma/dt = -\partial\mathcal{E}/\partial\mathbf{r}_\gamma$. Consequently, we gain access to the dynamics induced by the simplified model that I describe above. Moreover, the susceptibility of this approach to be trapped in local minima is ideal not only from an energetic perspective, but also from a numerical one: by taking incremental steps in a direction known to decrease the energy, any increases indicate a flaw and

In contrast to a numerical optimisation routine, gradient descent requires substantial explicit calculation. Moreover, the algorithm requires tuning in the step size and relative decrease in energy tolerance at which to decide the algorithm has terminated.

3.3.3 Deriving the gradient

The linearity of the gradient permits us to take the gradient term-by-term in equation (3.5). For an energy function $\mathcal{E}(\{\mathbf{r}_\gamma\})$ with cell normal vectors $\hat{\mathbf{n}}_\alpha$ approximated in terms of each cell's collar vertices, we find that the gradient $\partial\mathcal{E}_\phi/\partial\mathbf{r}_\gamma$ is given by

$$\frac{\partial\mathcal{E}}{\partial\mathbf{r}_\gamma} = \sum_{(\alpha,\rho)} \frac{-(\phi_{(\alpha,\rho)} - \phi_0)}{\sqrt{1 - (\hat{\mathbf{n}}_\alpha \cdot \hat{\boldsymbol{\alpha}}\rho)^2}} \left(\frac{\partial\hat{\mathbf{n}}_\alpha}{\partial\mathbf{r}_\gamma} \cdot \hat{\boldsymbol{\alpha}}\rho + \frac{\partial\hat{\boldsymbol{\alpha}}\rho}{\partial\mathbf{r}_\gamma} \cdot \hat{\mathbf{n}}_\alpha \right), \quad (3.14)$$

where for vectors \mathbf{a}, \mathbf{b} I denote $(\partial\mathbf{a}/\partial\mathbf{b})_{ij} = \partial\mathbf{a}_j/\partial\mathbf{b}_i$. When the cell normal vectors $\hat{\mathbf{n}}_\alpha$ are free variables, the first term in equation (3.14) is zero and we complete the calculation with

$$\frac{\partial\hat{\boldsymbol{\alpha}}\rho_j}{\partial\mathbf{r}_{\gamma i}} = \frac{\delta_{\gamma\rho} - \delta_{\gamma\alpha}}{|\hat{\boldsymbol{\alpha}}\rho|} [\delta_{ij} - (\hat{\boldsymbol{\alpha}}\rho)_i (\hat{\boldsymbol{\alpha}}\rho)_j], \quad (3.15)$$

where $\delta_{\gamma\rho}$ is an indicator function for equality between γ and ρ .

Equations equations (3.14) and (3.15) can be rearranged to efficiently calculate $\partial \mathcal{E} / \partial \mathbf{r}_\gamma$ with matrix multiplication by grouping terms dependent on γ , given an ordering of indices (α, ρ) :

$$\frac{\partial \mathcal{E}}{\partial \mathbf{r}_{\gamma i}} = \sum_{(\alpha, \rho)} \underbrace{(\delta_{\gamma\rho} - \delta_{\gamma\alpha})}_{A_{\gamma, (\alpha, \rho)}} \underbrace{\frac{-(\phi_{(\alpha, \rho)} - \phi_0)}{\sqrt{1 - (\hat{\mathbf{n}}_\alpha \cdot \vec{\alpha}\rho)^2} |\vec{\alpha}\rho|}} \underbrace{(\hat{\mathbf{n}}_\alpha - \vec{\alpha}\rho (\hat{\mathbf{n}}_\alpha \cdot \vec{\alpha}\rho))}_{B_{(\alpha, \rho), i}}, \quad (3.16)$$

where A and B are matrices with entries enclosed in the braces. The remaining gradient terms equations (3.17) and (3.24) can also be rearranged as matrix multiplications to speed up calculation.

As discussed in 3.2.2, an angle $\psi_{(\alpha, \beta; \rho, \sigma)}$ for a cell-cell interface depends in a piecewise constant way on the cells' normal vectors. While the angles are consequentially only piecewise differentiable, gradient descent makes reasonable the assumption that there will not be any discontinuities in \mathcal{E}_ψ for sufficiently small steps in the direction of the negative gradient. The below expression for $\partial \mathcal{E}_\psi / \partial \mathbf{r}_\gamma$ assumes that the sign of $\hat{\mathbf{n}}_\alpha \cdot (\vec{\alpha}\rho \times \vec{\beta}\sigma)$ remains constant.

$$\begin{aligned} \frac{\partial \mathcal{E}_\psi}{\partial \mathbf{r}_\gamma} &= k_\psi \sum_{(\alpha, \beta; \rho, \sigma)} \frac{(\psi_{(\alpha, \beta; \rho, \sigma)} - \psi_0)}{2\sqrt{1 - (\hat{\mathbf{n}}_{\rho\alpha\sigma} \cdot \hat{\mathbf{n}}_{\rho\beta\sigma})^2}} \left(\hat{\mathbf{n}}_{\rho\alpha\sigma} \cdot \frac{\partial \hat{\mathbf{n}}_{\rho\beta\sigma}}{\partial \mathbf{r}_\gamma} + \hat{\mathbf{n}}_{\rho\beta\sigma} \cdot \frac{\partial \hat{\mathbf{n}}_{\rho\alpha\sigma}}{\partial \mathbf{r}_\gamma} \right) \\ \hat{\mathbf{n}}_{\rho\alpha\sigma} \cdot \frac{\partial \hat{\mathbf{n}}_{\rho\beta\sigma}}{\partial \mathbf{r}_\gamma} &= \frac{\text{sgn}(\vec{\beta}\rho \times \vec{\beta}\sigma \cdot \hat{\mathbf{n}}_\beta)}{|\vec{\beta}\rho \times \vec{\beta}\sigma|} \left[\left(\frac{\hat{\mathbf{n}}_{\rho\alpha\sigma} \cdot \vec{\beta}\rho \times \vec{\beta}\sigma}{|\vec{\beta}\rho \times \vec{\beta}\sigma|^2} \vec{\beta}\rho \times \vec{\beta}\sigma - \hat{\mathbf{n}}_{\rho\alpha\sigma} \right) \right. \\ &\quad \left. \times \left((\delta_{\gamma\rho} - \delta_{\gamma\beta}) \vec{\beta}\sigma - (\delta_{\gamma\sigma} - \delta_{\gamma\beta}) \vec{\beta}\rho \right) \right] \end{aligned} \quad (3.17)$$

The gradient $\partial \mathcal{E}_{\text{sp}} / \partial \mathbf{r}_\gamma$ is given by the linear spring force

$$\frac{\partial \mathcal{E}}{\partial \mathbf{r}_\gamma} = k_{\text{sp}} \sum_{(\alpha, \rho)} (\delta_{\alpha\gamma} - \delta_{\rho\gamma}) \vec{\alpha}\rho \quad (3.18)$$

which is simply Hooke's law.

3.3.4 Forward integration

Integrating the gradient given by equations equations (3.14), (3.17) and (3.18) produces the dynamics of sheet bending as in figure 3.4. We can numerically integrate using gradient

descent with finite step size Δt and drag coefficient ζ .

$$\mathbf{r}_\gamma(t + \Delta t) = \mathbf{r}_\gamma(t) - \zeta \Delta t \frac{\partial \mathcal{E}}{\partial \mathbf{r}_\gamma}. \quad (3.19)$$

We can factor k_ϕ from each of equations (3.14), (3.17) and (3.18) to use step $\zeta k_\phi \Delta t$ in equation (3.19). With one of three energy constants only serving to affect the timescale, only the remaining two are free. I set $k_\psi/k_p h_i = 2^6$ and $k_{sp}/k_\phi = 5$ for the results in this work, though the results were not found to qualitatively differ significantly for small differences in these constants.

When treating the normal vectors $\hat{\mathbf{n}}_\alpha$ as free variables, we must solve modify our force equilibration algorithm to maintain the constraint that $|\hat{\mathbf{n}}_\alpha|^2 = 1$. An intuitive option is to step in the direction of the negative gradient and normalise the intermediate vectors at each step:

$$\mathbf{n}_\alpha(t + \Delta t) = \hat{\mathbf{n}}_\alpha(t) - \zeta \Delta t \frac{\partial \mathcal{E}}{\partial \hat{\mathbf{n}}_\alpha} \quad (3.20)$$

$$\hat{\mathbf{n}}_\alpha(t + \Delta t) = \frac{\mathbf{n}_\alpha(t + \Delta t)}{|\mathbf{n}_\alpha(t + \Delta t)|}. \quad (3.21)$$

Equivalently, since for a sufficiently small step size the nearest point on the constraint set $|\hat{\mathbf{n}}_\alpha|^2 = 1$ to $\mathbf{n}_\alpha(t + \Delta t)$ is unique, we may re-express equation (3.21) with $\hat{\mathbf{n}}_\alpha(t + \Delta t) = \arg \min_{|\hat{\mathbf{n}}_\alpha|^2=1} |\hat{\mathbf{n}}_\alpha - \mathbf{n}_\alpha(t + \Delta t)|$. Expressed in this way, we see that the update for $\hat{\mathbf{n}}_\alpha$ expressed in equations (3.20) and (3.21) is exactly the projected gradient descent algorithm and we expect it to converge [13]. Since the normal component of $\partial \mathcal{E} / \partial \hat{\mathbf{n}}_\alpha$ to the constraint set $|\hat{\mathbf{n}}_\alpha|^2 = 1$ at step t does not affect $\hat{\mathbf{n}}_\alpha(t + \Delta t)$, we can interpret equations (3.20) and (3.21) as taking a step in the direction of the tangent space to the constraint set that reduces \mathcal{E} the most.

⁶A value of 2 was chosen since each cell-cell interface is only counted once in equation (3.12), despite two collar microvilli being represented at each interface.

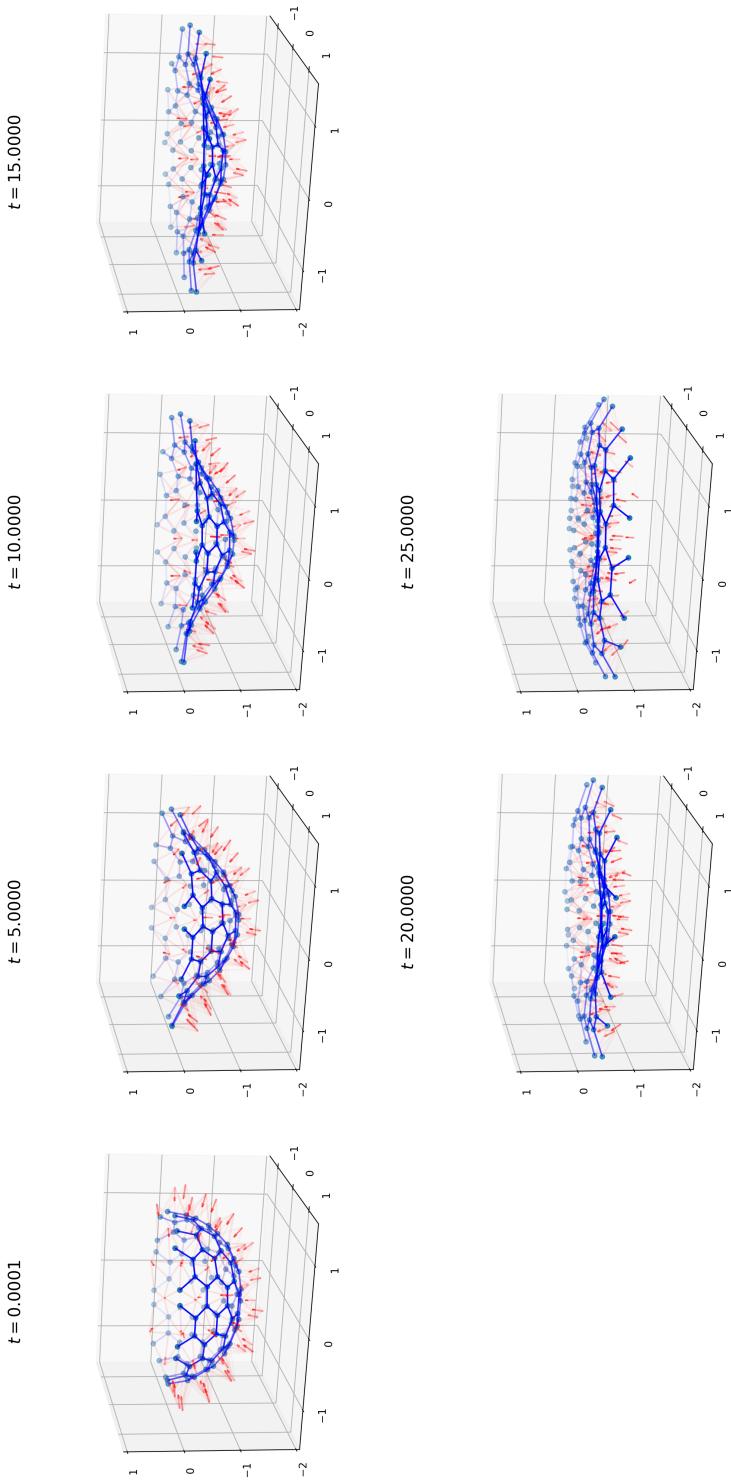


Fig. 3.4 Dynamics of sheet inversion in the transition from $(\phi_0, \psi_0) = (0.55, 0.65)$ to $(0.71, 0.41)$ demonstrating projected gradient descent using equations (3.19) to (3.21).

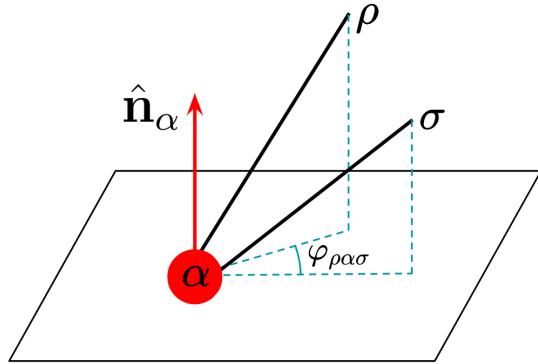


Fig. 3.5 Geometry for calculating $\varphi_{\rho\alpha\sigma}$.

While the forward integration works well for small sheets with simple graph topologies, I found that some equilibrium angles ϕ_0, ψ_0 caused some sheets to strain to such an extreme that collar vertices would cross through each other. The resulting increase in energy comes from the discontinuous sign function in the definition of ψ (equation (3.11)), and collar vertices cross over each other due to too large of a step size Δt . While adaptively decreasing the step size is a viable option, it would substantially slow the equilibration algorithm. Instead, I introduced an additional term to the energy \mathcal{E}_ϕ based on the angles $\varphi_{\rho\alpha\sigma}$ formed by each cell α and its adjacent pairs of collar vertices ρ, σ when projected onto the plane defined by the cell normal $\hat{\mathbf{n}}_\alpha$ (figure 3.5):

$$\mathcal{E}_\phi = k_\phi \sum_{(\alpha,\beta:\rho\sigma)} (\varphi_{\rho\alpha\sigma} - \varphi_{0\rho\alpha\sigma})^2, \quad (3.22)$$

where $\varphi_{0\rho\alpha\sigma}$ is an equilibrium projected collar-cell-collar angle for each triple. Each angle $\varphi_{0\rho\alpha\sigma}$ is set to the actual value that is evaluated at the initial sheet geometry. Unless specified otherwise, the constant k_ϕ is set to $0.3k_\phi$, which was qualitatively found to be a reasonable balance between preventing numerical instability and minimally interfering in the structure transition. It is worth noting that \mathcal{E}_ϕ has physical interpretation as penalising collars that do not extend radially outward relative to the cell's normal vector.

The angles $\varphi_{\rho\alpha\sigma}$ are calculated similarly to ϕ, ψ (equations (3.7) and (3.11)),

$$\varphi_{\rho\alpha\sigma} = \arccos [\vec{\alpha}\rho_{||} \cdot \vec{\alpha}\sigma_{||}], \quad (3.23)$$

where $\vec{\alpha}\rho_{\parallel}$ is the projection of $\vec{\alpha}\rho$ onto the plane defined by normal $\hat{\mathbf{n}}_{\alpha}$ and position \mathbf{r}_{α} :

$$\vec{\alpha}\rho_{\parallel} = \vec{\alpha}\rho - (\vec{\alpha}\rho \cdot \hat{\mathbf{n}}_{\alpha})\hat{\mathbf{n}}_{\alpha}.$$

The gradient of \mathcal{E}_{ϕ} is given by

$$todo \tag{3.24}$$

Note that

$$\mathbf{0} = \sum_{\gamma} \frac{\partial}{\partial \mathbf{r}_{\gamma}} \mathcal{E} \tag{3.25}$$

since all forces are generated internally. Moreover, equation (3.25) holds individually for each term in the energy (equation (3.5)), which is evident by the indicator functions in equations (3.14), (3.17), (3.18) and (3.24).

3.3.5 Exploring the energy landscape

With the ability to study discrete sheet equilibrium geometries and dynamics, we are prepared to evaluate a mechanism for *C. flexa* folding and inversion. Based on the two collar angle states observed in Brunet et al. [7], a reasonable model would be to assume relaxed-state equilibrium angles ϕ_{in}, ψ_{in} and a different set of active-state angles ϕ_{out}, ψ_{out} with instantaneous transition between the two states. The rapid change in individual cell behavior and expected gradual sheet shape change expected from opposing cell-cell interactions align with our expectations from observations Brunet et al. [7].

Although we cannot access true values for the equilibrium angles, modeling *C. flexa* sheets numerically provides the opportunity to explore the entire energy landscape. For sheets generated with a regular hexagonal lattice, we observe the expected diagonal valley where energy is minimised in the energy landscape since the sheet is expected to be flat along those pairs (ϕ_0, ψ_0) (figure 3.6) (section 3.3.1). Substantial sheet deformation and bending is evidently not sufficient to overcome the change in terms in the energy function. The increases in energy when the equilibrium angles are most disparate can be interpreted as collar microvilli stretching or compressing to accommodate sheet bending or a difference in the bending at each cell from the preferred state. For example, cells at neither the centre nor boundary in a bent sheet (figure 3.6, bottom-right or top-left) must contribute a positive

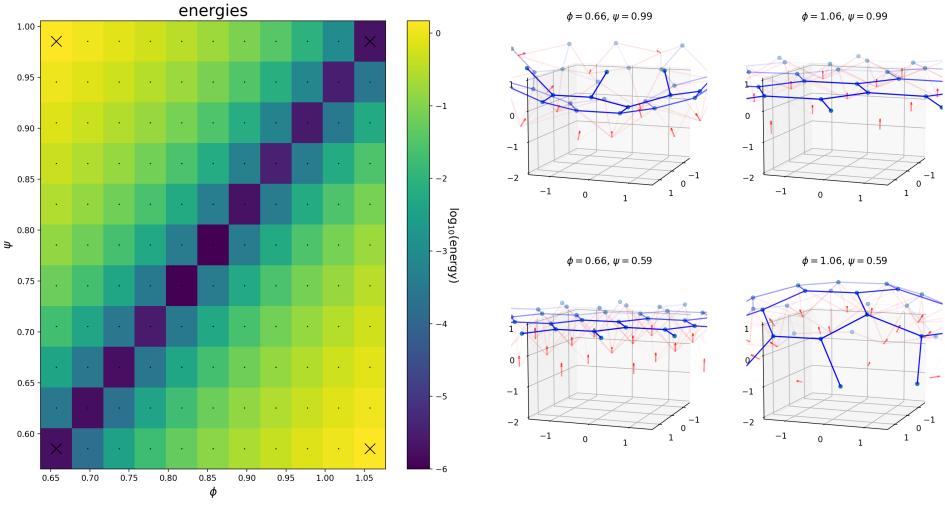


Fig. 3.6 Energy landscape of a discrete *C. flexa* sheet generated from a hexagonal lattice. Sheets displayed at the right correspond to the corners of the landscape indicated with white crosses. Energy along the diagonal is slightly above zero due to finite termination of gradient descent.

contribution to \mathcal{E} since they do not have the symmetric bending at all collar microvilli prescribed by equations (3.6), (3.12) and (3.13).

For graph topologies generated with an icosphere which contain topological defects, we observe a more rich energy landscape (figure 3.7). A small section of the icosphere (points with polar angle greater than $3\pi/5$ with respect to vertical axis) gives an energy landscape with two diagonal valleys corresponding to flagella-in and flagella-out sheets (upper and lower, respectively). That there are two valleys, rather than the one valley along $\phi_0 = \psi_0$, is interpreted to be the result of topological defects in the lattice structure that are essential to the construction of the icosphere. The two energy valleys achieve similar values for the minimum sheet energy, suggesting there is not necessarily any energetic preference to either state in this model. The positive and negative sheet curvatures respectively, in the convention of chapter 2 is consistent with how we expect ϕ_0 and ψ_0 to induce spontaneous curvature (equation (2.8)). The continuity between the two valleys indicates that sheets are stably able to flatten and the minimum energy structures continuously transition between flagella-in and -out structures. The landscape was found to differ negligibly when an inverted sheet was used to initialise the simulations.

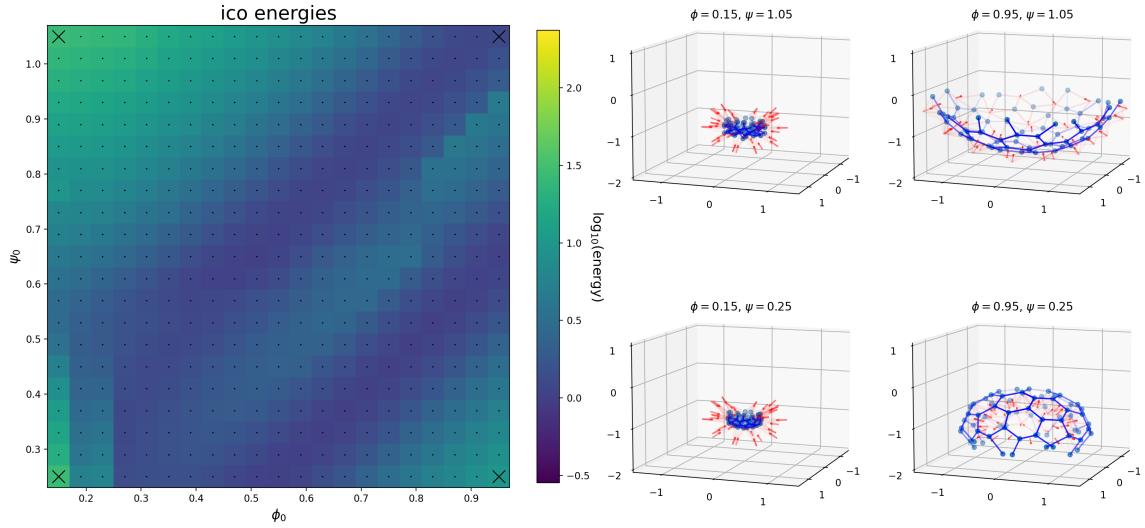


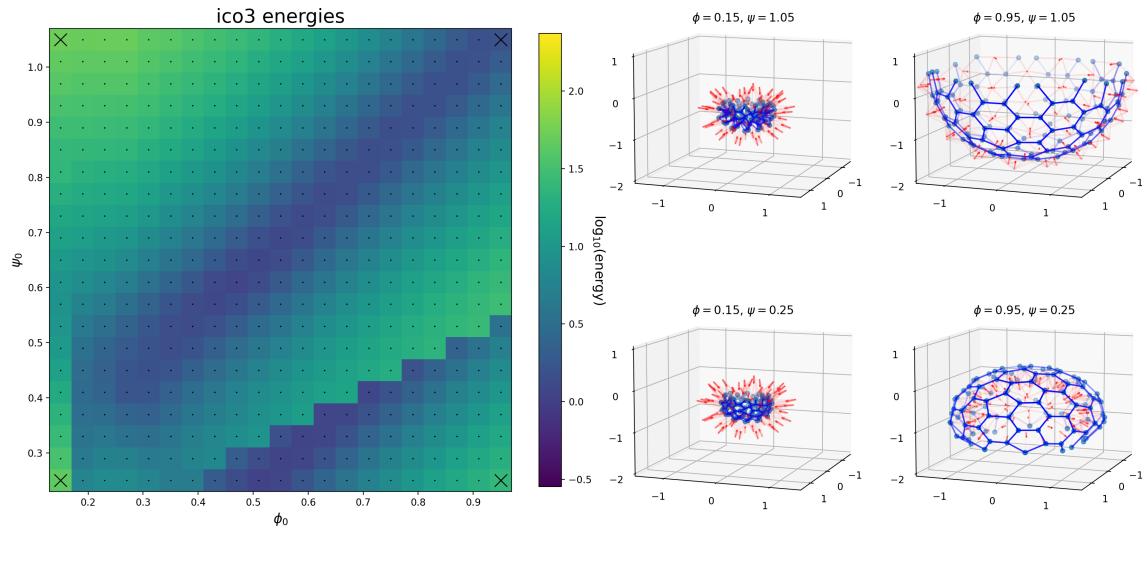
Fig. 3.7 Energy landscape of a discrete *C. flexa* sheet generated from a thrice-subdivided icosphere section. Style as in figure 3.6. The icosphere was generated using code from [11].

3.3.6 Sudden inversion

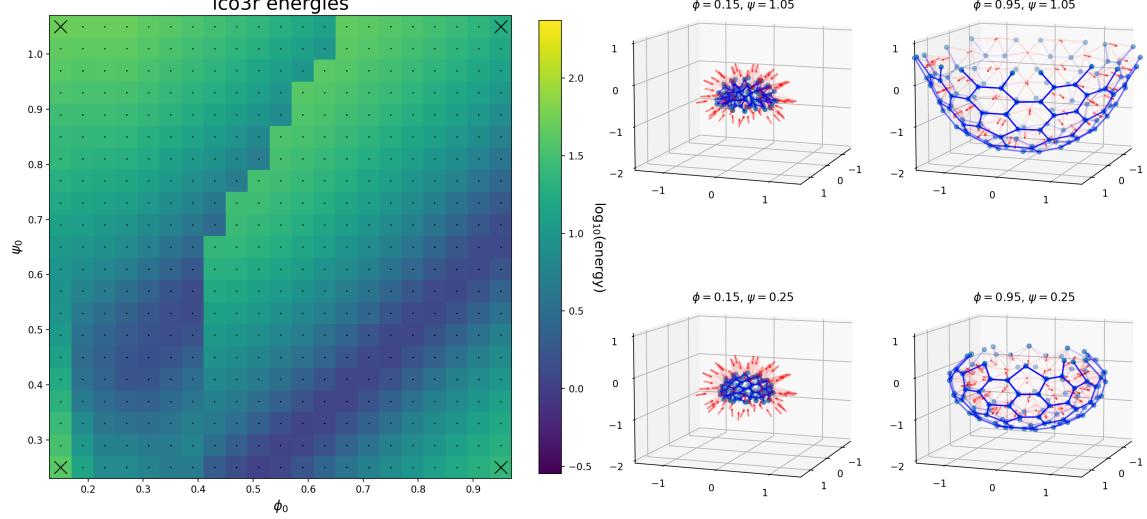
Adding more cells by taking more cells from the icosphere (with polar angle at least $2\pi/5$) again produces two energy valleys corresponding to flagella-in and -out sheets (figure 3.8). In a larger sheet, however, we identify a discontinuity when beginning from a flagella-in sheet and changing parameters to achieve inversion (figure 3.8a) as in figure 3.4. The discontinuity implies that equilibrated sheets flagella-in sheets there achieve a local energy minimum, but that a global minimum may be achieved instead by taking a flagella-out sheet with the same parameters. Indeed, when using flagella-out sheets as initial conditions, the complete flagella-out energy valley is apparent and the discontinuity shifts to show the flagella-out to -in transition (figure 3.8b).⁷ Note that the sheet geometries at the landscape corners in figure 3.8 are the same, confirming inversion and the existence of unique minima in the landscape.

As we are interested in the bistability and transition in *C. flexa* sheets, we are concerned with the global energy minima for both flagella-in and -out sheets. In figure 3.9, the minimum energy geometries from the landscapes of figure 3.8 are shown with an indication which

⁷The large discontinuity along $\phi_0 = 0.41$ is a consequence of the simulation parallelisation. All landscape plots shown were simulated by first equilibrating at the landscape centre ($\phi_0 = 0.55, \psi_0 = 0.65$). The row $\psi_0 = 0.65$ was simulated by using this as an initial condition to consecutively equilibrate to a larger or smaller value of ϕ_0 . Sheets were then simulated in columns from the central row for increasing or decreasing ψ_0 . The vertical discontinuity indicates where the initial row simulations first transitioned, such that the sheets with $\phi_0 = 0.39$ in figure 3.8b began with a flagella-in sheet.



(a)



(b)

Fig. 3.8 Energy landscapes for (3.8a) flagella-in and (3.8b) flagella-out sheets of *C. flexa*. The color scaling is the same in both images. The landscapes for a smaller segment of the icosphere were qualitatively similar.

of the two plots the conformation came from. The gloabl energy minima show two clear contiguous minimum energy valleys, as in figure 3.7.

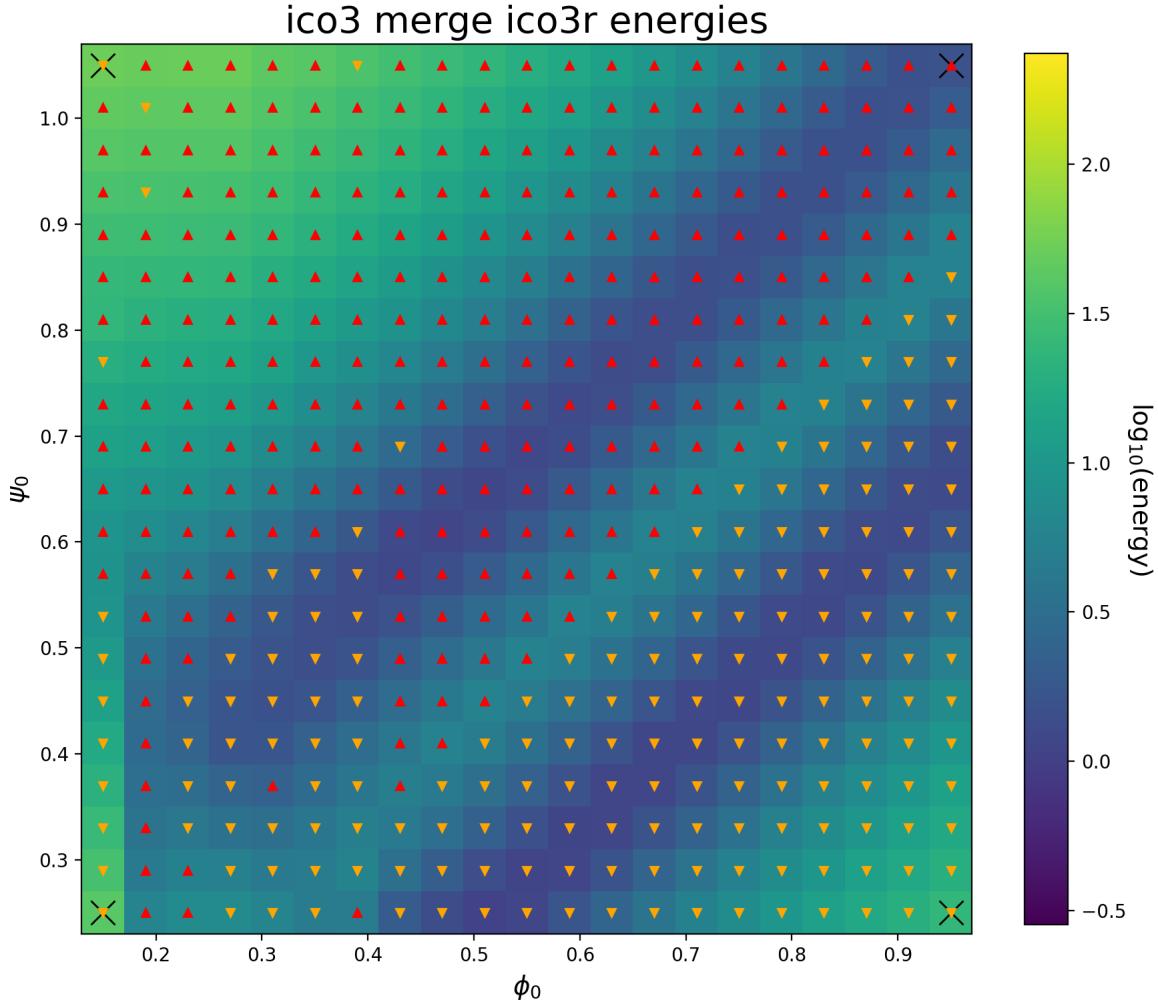


Fig. 3.9 Minimum energies the two landscapes shown in figure 3.8. Values pulled from figure 3.8a are denoted with red triangles and figure 3.8b with orange triangles.

3.3.7 Inversion dynamics

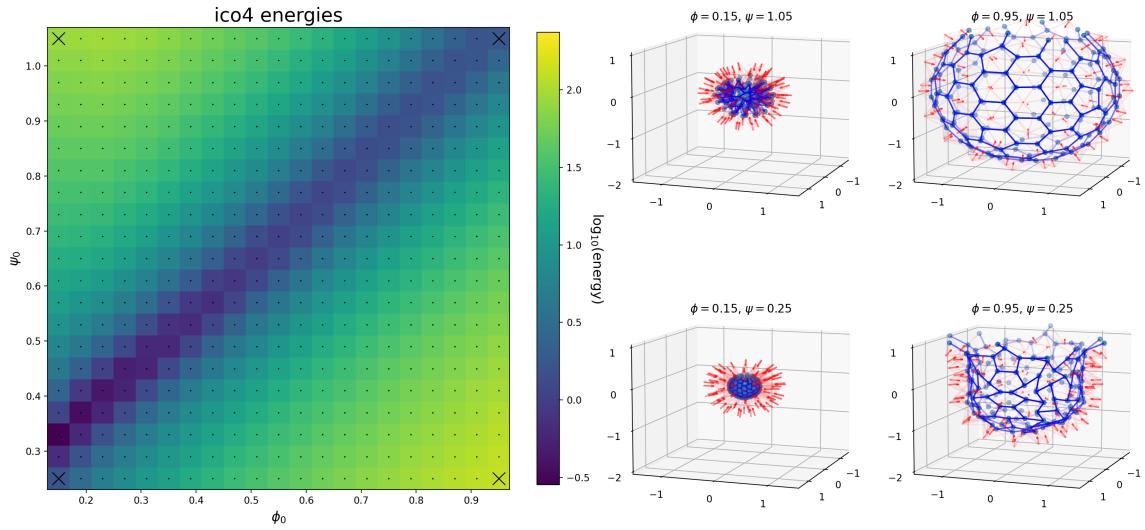
Informed by the transitions shown in the figure 3.8 landscapes, we ask what the inversion dynamics look like in this model. Figure 3.4 shows the inversion from flagella-in to flagella-out by a single, sudden change in parameters. Unlike the filament model studied in section 2.1

(figure 2.2) and the small sheet in section 3.3.5, large sheet inversion in the discrete model features temporary flattening through stretching at the edges. The propagation of the change in curvature from the sheet boundary to the centre results in the *rolling over* effect described in section 2.1 that is missing from those less detailed and smaller sheet descriptions.

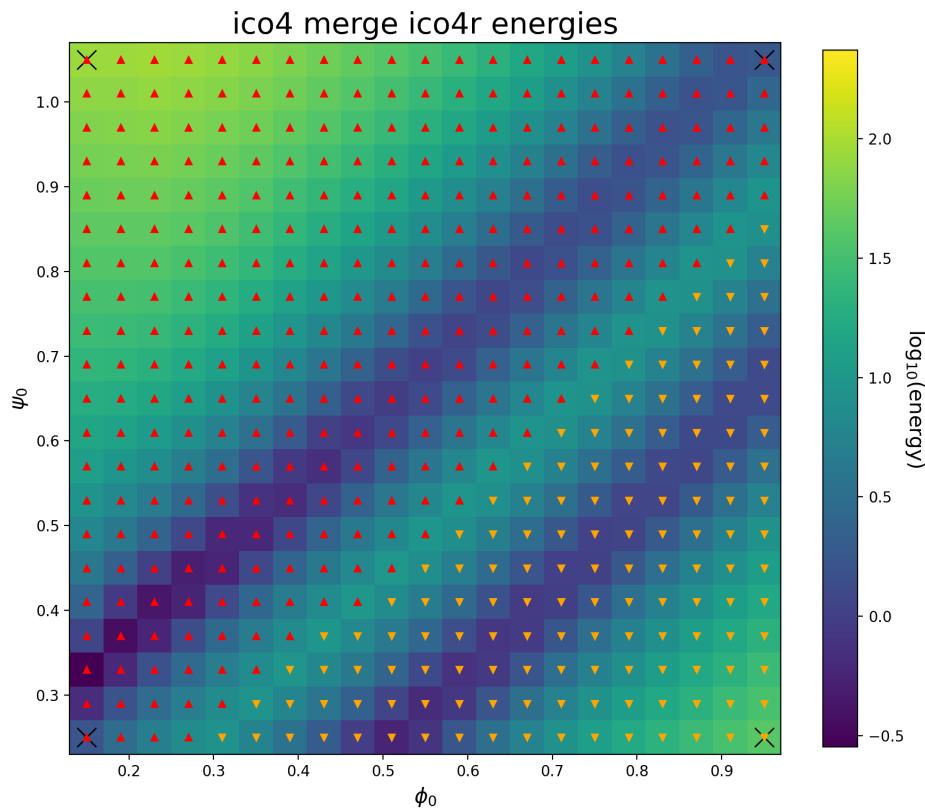
The continuities in figure 3.8 and inversion dynamics indicate that the topology of the cell-cell connection graph is key to producing bistability, where both flagella-in and flagella-out states are achievable as local energy minima for the same model parameters. In contrast, a sheet without topological defects (figure 3.6) or a small sheet with fewer cells at the boundary (figure 3.7) readily inverts between states with similar energies.

Taking this further, we expect that some sheet topologies may be so restrictive at the boundary or elsewhere that inversion is completely prohibited for sufficiently high k_{sp} . Taking most of an icosphere to generate the sheet topology results in a complete inability to invert for the same energy constants used in figures 3.6 to 3.8. Instead, sheets retain their orientation always and experience substantial stress when the equilibrium angles prescribe a curvature that cannot be achieved in the present sheet orientation (figure 3.10). The flagella-out landscape is qualitatively similar with a valley in the same location as ???. The combined landscape of flagella-in and -out sheets (figure 3.10b) again shows the two valleys, now separated by an insurmountable energetic barrier.

It is clear that there may be a substantial energetic barrier to reach that state induced by collar-collar adhesion forces and collar stiffness. While this energetic barrier can be overcome by further pushing the equilibrium angles into a region that favors the opposite curvature (figure 3.8) in simulations, *C. flexa* cells appear to act in exclusively one of two pre-determined states. There is no evidence to suggest changing collar properties, so we are led to predict that changing cell sheet topology is the factor which enables inversion.



(a)



(b)

Fig. 3.10 (3.10a) Energy landscape for large flagella-in sheets. The landscape for flagella-out sheets was quantitatively similar, with the minimum energy valley shifted downward as in figure 3.8b. (3.10b) Minimum energies for flagella-in and -out large sheets. Style as in ??.

Chapter 4

Discussion

The modeling done here offers several perspectives to study the mechanics of sheet shape and inversion in *Choanoeca*. We see that the discrete, lattice structure of the sheet makes the sheet’s intrinsic curvature possible, and the sheet’s curvature emerges in response to the lattice structure. The presence of topological defects gives a tight ring of cells at the sheet boundary, which presents an energetic challenge during inversion. The models that I built make it clear that stretching plays a major role in the dynamics of sheet inversion, and they distill the essential mechanics of *C. flexa* colony shape to only two parameters. This discrete model of sheet inversion supports the hypothesis that *C. flexa* inverts using an instantaneous active change of collar microvillus preferred shape.

4.1 *C. flexa* geometry

The small curvature in *C. flexa* colonies permit them to drive strong flows by aligning their apicobasal axes close to parallel while simultaneously producing two stable shape equilibria. This contrasts with the rosette colonies of *S. rosetta*, which are known to have morphology inefficient for driving feeding flows [28].

Connections via an extracellular matrix are known to produce choanoflagellate colonies as disks, cups, or branched trees with flagella splayed out depending on the material properties [32]. In each of these, that the ECM connects cell bodies prevents cells from imparting a force on each other through direct contact. Nevertheless, a substantial amount remains to be learned about the collar-collar connections that facilitate *Choanoeca* colony cohesion. For collars to splay as they do in individual cells, they must not adhere to each other; a small azimuthal torque would otherwise easily cause several microvilli to join, interfering with flagellar beating.

Choanoeca is remarkable in the common orientation of all cells in its colonies. This is made possible by the absence of any discernible extracellular matrix [35, 7], which would otherwise be expected to confine colonies to some other morphologies [32]. Apart from the mechanical implication of facilitating inversion, connections at the collars that align cell apicobasal axes drive the strongest flows through collars in a numerical model [28]. While it is enticing to speculate about *Choanoeca* evolution based on its colonies' ability to change shape, it is worth keeping in mind that there may have been other pressures for evolving as the species did.

When thinking about *C. flexa* in the context of multicellularity, we should not overlook the simplicity by which it achieves large-scale geometric changes. While we can develop increasingly complex models by introducing collar filament bending, tension and stress at the collar filaments' bases, or the effects of the contractile ring, my work demonstrates that a coarse description of individual cells is sufficient to explain the behavior that we observe in colonies. Compare this with *Volvox*, which uses connections and communication between cells to control its inversion. One might imagine that the complexity of a molecular pathway for a single cell to exhibit phototaxis or regulate feeding/swimming efficiency could easily exceed the ring contraction as currently understood in *C. flexa* [7].

4.2 Discrete cell sheet topology

The discrete model of *C. flexa* demonstrates that deviations from a hexagonally packed sheet at as few as one cell are sufficient to induce substantial bending. This result is consistent with that described in Seung and Nelson [51] for planar lattices, albeit through a different mechanism where stretching and bending are closely related. Indeed, topological defects induce buckling and non-local inability to satisfy equilibrium conditions, yet these defects are also vital to achieving large-scale sheet curvature.

Physical reasoning suggests that interfaces between cells would be equidistant from the cell bodies, since each cell is assumed to be identical and the forces must balance at equilibrium. Imaging supports this idea (figure 1.1), though any imaging requiring fixation may affect the collar stiffness or preferred curvature. That the collar microvilli at the boundaries form an arc around the apicobasal axis suggests that they freely take angle ϕ_0 at the base and do not contribute meaningfully to the energy (figure 1.1d). This validates the treatment of boundary collar vertices used in the discrete model.

Leadbeater [35] describes that in *C. perplexa* colonies, each cell is typically adjacent to six others. Given the strong *Choanoeca* sheet curvatures and known association between

Gaussian curvature and topological defects in lattice structure (section 2.2.3) [50, 51], it is reasonable to expect that many cells have more or fewer than six neighbors. Even in a small section of the colony figure 1.1c, several defects may be readily identified.

4.3 Extensions

Both the continuous and discrete models developed make clear that azimuthal stretching in curved sheets is critical in *C. flexa* inversion, especially by introducing an energetic barrier. With even relatively large-area sheets observed inverting readily in culture (collar diameter relative to sheet major-axis diameter as low as $\sim 20\%$), collar-collar interfaces must either be able to substantially stretch or connections must break.

A model for *Choanoeca* colony growth may help understand the origins and distributions of topological defects in the lattice structure. Given the possible division strategy of these cells (figure 1.2) and the idea that other choanoflagellate colony cells divide without synchronisation [32], we might build a model by spontaneously splitting cells into pairs of adjacent daughter cells. The goal of a colony growth model is two-fold. First, a model for colony growth may explain the origin of topological defects in *C. flexa* colonies. We would obtain graph topological statistics that are comparable to experiments to determine if this method of colony growth is accurate or if another method produces the observed topological defects. Second, such a model may cause the cup-like sheets observed, rather than sheets with other complex geometries. Moreover, the distribution of defects over the sheet may produce non-icosahedral or -spherical equilibrium geometries, as large *Choanoeca* sheets tend to be ellipsoidal rather than spherical [35, 7]. It remains to be resolved how colonies form without buckling at sheet boundary: uniform, isotropic cell division would give proportionally scaling area and boundary length, but preferred sheet curvature would cause buckling with too large of a boundary.

An alternative theory for *Choanoeca* colony growth is via aggregation [18], though choanoflagellate colonies have only been observed to form by clonal division [15, 1, 65]. Sequential cell adhesion at the boundary would resolve buckling at the edges due to uniform, isotropic growth. Moreover, aggregation resolves the issue that there are a discrete number of microvilli at cell-cell interfaces. Hence, the interfaces cannot be divided between daughter cells indefinitely. Recent evidence from *S. rosetta* points that several processes involved in maintaining the colony ECM are involved in preventing spurious aggregation [62], indicating that aggregation is specifically disfavored in other choanoflagellates. Colony formation by aggregation is unprecedented in our understanding of choanoflagellates, and of course

comes with the organisational challenge of producing a single consistent orientation over the entire sheet.

While the structures used in this work had discrete symmetry around an axis, *C. flexa* sheets observed experimentally may have substantial irregularities both in the bulk and at the boundary. Several images in Brunet et al. [7] show uneven sheet boundaries, resembling crenellations of a castle wall (figure 4.1). As suggested in section 3.3.7, a sheet with excessively restrictive lattice topology may be able to achieve inversion by a temporary change in its cell-cell connections. An extreme example of inversion through topological change is performed by *Volvox* embryos, which invert by creating a hole in their sphere and pass the entire sheet through the hole [23]. Type-A *Volvox* inversion forms this hole by making four lips and peels them back to begin inversion at the boundary, similar to how inversion proceeds in figure 3.4 [60]. Even besides boundary ruggedness, asymmetry in the sheet may promote the initiation of inversion at one region of the boundary while it is not yet possible elsewhere.

Leadbeater [35] described that large *C. perplexa* sheets may take irregular shapes such as ribbons. Either as a consequence of tearing during inversion or itself causing tearing during inversion, such unusual geometries may be involved in colony division. If colonies tear, we would not expect them to fully separate since a single connection at the boundary of either colony fragment would not hinder inversion. Consequently, it is possible that the weight of colony fragments themselves in the flagella-in state (with resulting incompatible drag tensors) or the strong collective flows driven by separate colony fragments in the flagella-out state are responsible for separating colony fragments. If entirely physically facilitated, colony division is likely to substantially involve both cell-cell connection lattice structure and flows.

An underlying assumption throughout this work is that *Choanoeca* colonies consist entirely of identical cells, both in genetics and morphology. Recent findings in *S. rosetta* suggest that choanoflagellates are capable of spatial differentiation within colonies [33, 45]. Different properties throughout the cell-cell connection network, especially different preferred collar shapes due to cell body morphology or filament mechanics, substantially affect inversion dynamics. Most substantial would be if some adjacent cells do not form adhesive contact at all, in contrast to our current interpretation of *Choanoeca* colony imaging [7].

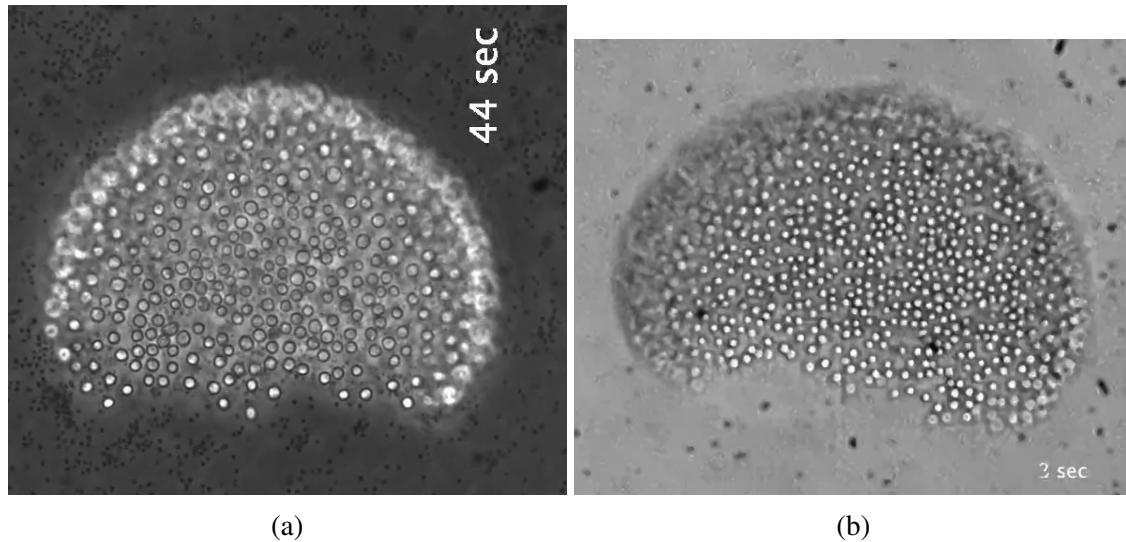


Fig. 4.1 *C. flexa* sheets with ragged boundaries. From Brunet et al. [7]. Reprinted with permission from AAAS.

4.4 Multicellularity and *C. flexa*

Ultimately, multicellularity in *Choanoeca* appears to be the result of a tradeoff between a decrease in the flow efficiency of individual choanoflagellate cells and the ability of the colony to invert. Inversion is highly dependent on the sheet structure and its lattice, which may itself change to facilitate inversion and potentially colony division. A more complete experimental characterisation of *Choanoeca* colonies would further inform the models developed here and clear up the connection between lattice topology, inversion, colony formation, and colony division. Nevertheless, the multicellular mechanics of *C. flexa* are crucial to its inversion and the behavior that inversion make possible.

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Appendix A

Continuous energy functional derivation

Chapter 2 expresses an integral to evaluate the energy density at a given point in terms of constants a, b, c, d, e .

$$\begin{aligned} \int_{-\pi}^{\pi} (\phi - \phi_0)^2 d\theta &= \int_{-\pi}^{\pi} \frac{(a \cos^2 \theta + b \sin^2 \theta + 2c \sin \theta \cos \theta + d)^2}{(a \cos^2 \theta + b \sin^2 \theta + 2c \sin \theta \cos \theta + e)^2} d\theta \\ &= \left\{ -\sin 2\theta [a^2(-4bc\theta - 4ce\theta + d^2 - 2de + e^2) \right. \\ &\quad + b^2(4ce\theta + d^2 - 2de + e^2) + 4bc\theta(e^2 - c^2) + 4c^2(d - e)^2] \\ &\quad + 2(a + b + 2e) [c^2\theta(b - a) + \theta(a - b)(a + e)(b + e) - c(d - e)^2] \\ &\quad \left. + 2\theta(a - b)^2 \cos(2\theta)(a(b + e) + e(b + e) - c^2) \right\} \\ &/2 [(a - b)(a(b + e) + e(b + e) - c^2) \\ &\quad ((a - b)\cos(2\theta) + a + b + 2c \sin(2\theta) + 2e)] \\ &+ \frac{(e - d)(a(4b + d + 3e) + bd + 3be - 4c^2 + 2de + 2e^2)}{2(a(b + e) + e(b + e) - c^2)^{3/2}} \\ &\tan^{-1} \left(\frac{-(b + e) \tan \theta - c}{\sqrt{a(b + e) + e(b + e) - c^2}} \right) \end{aligned}$$

evaluated at $\theta = -\pi, \pi$.

