Cell geometry, microtubule anchoring and anisotropic dynamic instability: challenges and solutions to transverse cortical array organization

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Abstract

The self-organization of cortical microtubules within plant cells is an emergent phenomenon with important consequences for the synthesis of the cell wall, cell shape, and subsequently the structure of plants. Mathematical modelling and experiments have elucidated the underlying processes involved. There has been recent interest in the influence of geometric cues on array orientation, be it direct (cell shape) or indirect (tension in the membrane). However, the mechanical influence of membrane curvature on these elastic filaments has largely been ignored. A previous model was proposed to describe how the anchoring process may control the shape of individual microtubules seeking to minimize bending on a cylindrical cell. We implement this process into a model of interacting microtubules and find the cell curvature influence should be significant: the array favours orientations parallel to the direction of elongation rather than the expected transverse direction. Even without elasticity, the geometry of large cells hinders robust microtubule organization. These results suggest the necessity of additional processes to overcome these factors. We propose a simple model of orientation-dependent catastrophe in the context of cellulose microfibrils impeding microtubule polymerization and find a moderate impedance to be sufficient to generate transverse arrays despite the geometric influences.

1 Introduction

Plant morphology depends on microscopic-level processes within each cell. In particular, microtubule (MT) polymers organize along the cortex of cells to guide cellulose deposition [6]. In turn, the orientation of the cellulose microfibrils provides a mechanical constraint determining the direction in which the cells can elongate. Collectively, this contributes to the macroscopic shape of plant

organs. The organization of cortical microtubules (CMTs) is of particular interest because plants lack the organizing centres present in animal cells. Instead, self-organization is observed to arise from various factors including MT-MT interactions along the cortex [13], modes of MT nucleation [8, 31], cell geometry [3], and the regulation of MT-associated proteins involved with said processes [4, 24, 50, 29, 32]. Due to the difficulty of studying each aspect *in vivo*, quantitative models have been effective at testing hypotheses relating to each factor in isolation. The most direct method of modelling is agent-based, where the agents are MTs represented as curves interacting on the two-dimensional cortex. These models have proven helpful in analyzing CMT organization, and remain as a popular tool to explore the importance of each process involved [35, 16]. Investigating the fundamental principles behind array organization, these models have shown the importance of dynamic instability parameters (polymerization speed, depolymerization speed, catastrophe rate, etc.), and the various resolutions of MT-MT interactions (entrainment, crossover, collision-induced catastrophe) [40, 2, 44]. Subsequently, more sophisticated MT mechanisms such as MT-dependent nucleation (whereby MTs nucleate from existing MTs) and severing (whereby an existing MT is severed) have been implemented to demonstrate their roles in increasing (or decreasing) organization [2, 12, 15, 11]. More recently, models of protoxylem patterning have revisited the role of MT-dependent nucleation where is facilitates banded MT formation[38, 23, 22, 36].

One important question is how the CMT array in diffusely expanding cells, which expand uniformly along one axis, orders in a manner transverse (perpendicular) to the axis of cell elongation during its rapid growth phase. Previous work [3] found that MT-stabilizing proteins known as CLASP localizes to the sharp top/bottom edges of the short root-division-zone in Arabidopsis, facilitating the passage of MTs around these edges. In CLASP knockouts, MTs in those same cells underwent a trasition to depolymerization upon reaching these edges, a process known as edge-induced catastrophe (EIC). Said knockouts are observed to have highly ordered transverse arrays not seen in the wild-type case. *In silico* testing of EIC found that it was indeed sufficient to induce transverse arrays [3]. Thus, there was a general consensus that edge-induced catastrophe is responsible orienting CMT arrays in the transverse direction [16].

To understand the very basic factors governing CMT organization, the early models made simplifying assumptions on the cellular geometry and MT trajectories; that cells were planar or polyhedral and MTs grow along locally curvature-minimizing curves, known as geodesics. We will refer to the curves traced by the MT body as the MT shape. In practice, this results in MTs growing along straight lines [2, 4, 7], or helices along a cylindrical surface [15, 43, 38]. Although more recent work has incorporated fluctuating MT shapes with finite persistence lengths [30, 22], these are similar in that the MTs deflect without bias and travel along geodesics on average.

Building on these foundations, there has been recent interest in modelling the role of complex cell geometries on MT organization and CMT response to mechanical stress in the cell wall [35, 7, 30, 10, 18, 27]. This poses new modelling challenges: from generalizing previous methods to complicated shapes, to

reconsidering the validity of previous model assumptions when applied to this new context. Cell geometry can impact organization in different ways: modifying the connectivity of regions, introducing sharp corners, and the influence of local curvature on MT bending. In the direction of the first two geometric influences, researchers have studied MT growth on complex geometries while keeping with the geodesic assumption [30, 7]. Thus far, all models of interacting MTs ignore the effects of membrane curvature on the bending of these relatively stiff polymers. We present the first model to explore the question: could curvature effects impact CMT organization? If so, how can this be regulated by the cell?

The effect of curvature was first considered by Lagomarsino et al. [25] in the hypothetical case of individual MTs with lengths on the scale of a whole cylindrical cell. Their predictions of MTs favouring low curvature configurations were generally consistent with their *in vitro* experiments of MTs confined to the volume of microfabricated chambers. *In vivo*, MTs are constrained to the cortex by proteins anchoring the MT with the membrane, although the exact proteins responsible are largely unknown [18]. This effectively constrains MTs in the direction of growth, prohibiting lateral movement. In a cylindrical geometry, MTs tend to favour the longitudinal direction, corresponding the flattest direction. This is indeed observed in the non-expanding "shank" regions of tip-growing cylindrical cells such as root hairs and pollen tubes in contrast to the transverse CMT arrays are typically observed in cells that are elongating by diffuse expansion [47]. In an ideal case from the perspective of bending energy, MTs would grow parallel to the long axis. However, if anchored at in a manner non-parallel, the entire MT cannot laterally rotate into this configuration. Rather, their unclamped growing tips would deflect away from the geodesic path prescribed by the initial anchored angle toward the curvature-minimizing direction. The question of how MT lengths affect their shape as compared to the typically-assumed geodesics was further studied by Bachmann et al. [5]. In particular, it was found that the unanchored MT tip may deviate – bifurcate in special cases – from geodesics if the inter-anchoring distance is large enough. Conversely, the geodesic shape may be preserved in the limit of continuous anchoring. This is seen in the top of Fig. 1, where energy minimizers of various lengths are compared to a geodesic curve of the same nucleation angle. Subsequently, a minimal model of anchoring was proposed by Tian et al. [42] to describe MT shapes, and how this may be regulated by mechanics induced by randomly placed anchors. In the model of [42], there are two main factors: the anchoring rate and the total MT length. This can be seen in the bottom of Fig. 1: the larger the anchoring rate (red, green, and black in increasing order), the smaller the MT segments and therefore the smaller the deflections from geodesics. The cumulative effects of many segments may lead to a larger deflection regardless of the small anchoring lengths, making net effect subtle to predict. This is shown by the final positions of each MT tip. In vivo, MTs may vary in length due to dynamic instability and their various interactions. Thus, it remained difficult to deduce whether this effect on individual MTs influenced array organization without a full simulation of interacting MTs. In this work, we present a simulation of interacting and bending MTs to examine the

influence of MT-curvature minimization. We then propose a simple model for a transverse biasing mechanism that may counteract this bending influence.

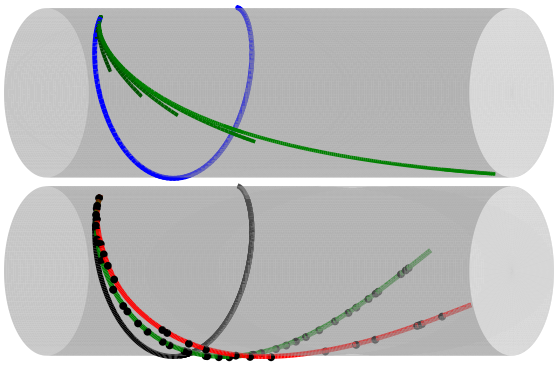

Figure 1: The modelling of microtubule bending on a cylinder. All curves represent MTs with the same initial position and growth direction. The MTs are clamped at their base, fixing the initial tangential angle and prohibiting lateral movement of the entire MT. Top: a comparison of MT shapes that minimize the bending energy Eq. 1 (green) and corresponding geodesics (blue) for MTs anchored and clamped only at one end. Geodesics on a cylinder are helices of a constant pitch, determined by the initial angle of nucleation. In this case, the initial angle is nearly transverse to the long axis. The differing shades of green represent the shapes corresponding to energy minimizers of differing lengths, with the longest green MT having the same length as the geodesic MT. Longer MTs tend to deviate more from the geodesic due to the preference to align parallel in the direction of lowest curvature, the longitudinal direction. Bottom: MT shapes of the same length incorporating the anchoring model in [42], with black dots indicating anchoring positions generated with the stochastic model. In this model, the geodesic (black) is the infinite rate limit, covering the entirety of the MT in anchors. The green MT is simulated with estimated parameters (cylinder radius, anchoring rate, and polymerization speed) used in Table 1, and red with an arbitrarily chosen lower anchoring rate.

2 Methods

Sections 2.1-2.3 summarize aspects of the model that have been described in previous works, namely, MT-MT interactions, parameters used, the anchoring process, and order parameters used in analyzing CMT organization. The remainder of Section 2 describes new aspects of the model: the inclusion of curved MTs along with the approximations made, the additional MT interactions introduced, and a model of cellulose feedback with the CMT array.

2.1 System Parameters

We base our system parameters on those used in previous studies [43, 12, 44] to provide a good basis for comparison. These are idealized or estimated from

experimental sources. In dimensional form, this is shown in Table 1. In particular, the dynamic instability parameters are those measured from Tobacco BY-2 cells [46], which have a geometry close to that of a cylinder and are diffusely expanding. This is as opposed to Arabidopsis cells that tend to be packed into tissues and organs, giving a prism shape with flatter faces and sharp edges.

We used the two-state model of dynamic instability: growth and shrinkage of the plus end with pause states excluded. These dynamic instability parameters are from [46]. There is constant depolymerization of the minus end, with the rate given by [39]. For simplicity, we included isotropic nucleation without MT-dependent nucleation. We set the default nucleation rate as in [15]. Severing is absent. The anchoring rate parameter (described later), k^*_{on}, was estimated by Allard et al. [1] in Arabidopsis cells.

Parameter	Description	Value
v_g	Plus end growth speed [46]	0.08 μms^{-1}
v_s	Plus end shrinking speed [46]	0.16 μms^{-1}
v_t	Minus end shrinking speed [39]	0.01 μms^{-1}
r_n	Nucleation rate [15]	0.001 s$^{-1}\mu$m^{-2}
r_{obs}	Observed catastrophe rate (see Sec. 2.7) [46]	0.0045 s^{-1}
r_r	Rescue rate [46]	0.007 s^{-1}
$\Delta\varphi^*$	Critical angle [13]	40°
p_{cat}	Catastrophe probability [13]	0.5
p_{zip}	Zippering probability [13]	1
R	Cylinder radius	20 μm
L	Cylinder length	$2\pi R$ (long) or $2R$ (short)
$p_{\text{edge-cat}}$	Probability of edge-induced catastrophe	1
k^*_{on}	Default anchor rate [1]	0.0057 s$^{-1}\mu$m^{-1}
l_d	Default mean first-anchoring length (dimensional)	0.24R

Table 1: Parameters used in the simulation in dimensional form. Parameters are segregated in collections of rows from top to bottom describing: dynamic instability and nucleation rates, MT-MT interactions, cell geometry, and MT anchoring. We denote the default anchoring rate as k^*_{on}, to distinguish from varying values of k_{on} used in the simulation. Catastrophe-inducing boundaries are at the top and bottom of the cylinder. Parameters from experimental measurements have been rounded, and others are estimated from casual observations.

The domain is first set such that the cylinder height equals circumference ($L = 2\pi R$). We refer to this as the long cylinder case, because the height is π times the diameter. Unravelled, the cylinder surface is square. This allows for a direct study of curvature influence while maintaining a biologically relevant geometry. In addition, we simulate on a shorter cell where the height matches the diameter. Because elastic MTs will tend toward the longitudinal direction, we expect a competition between the longitudinal bias from the bending influence and transverse bias due to edge-induced catastrophe. To examine whether

edge-induced catastrophe remains sufficient in generating transverse CMTs, we implement a strong catastrophe condition: 100% catastrophe along the edges of the cylindrical caps. For this reason, we omit the study of MTs on the cap surfaces.

2.2 Microtubule Interactions

Upon a collision of an incident MT (Fig 2, black) and a barrier MT (Fig 2, grey), there are three possible outcomes: entrainment (incident MT follows the barrier MT), collision-induced catastrophe of the incident MT, or crossover (incident MT continues past the barrier MT) without severing. The outcome is determined by the angle of incidence: entrainment if below the critical angle $\Delta\varphi^*$, and a 50% chance for catastrophe or crossover otherwise. This choice of critical angle is consistent with the majority of models thus far [2, 3, 12, 15, 43, 30], with some models differing in the probability of catastrophe [15, 30].

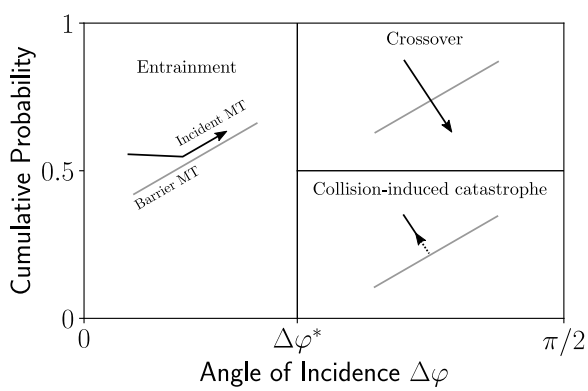

Figure 2: Illustration of collision resolution probabilities used. For angle of incidences less than $\Delta\varphi^* = \frac{2}{9}\pi$ (40°) between the barrier MT (grey) and the incident MT (black), the result is always entrainment. For larger angles, there is a 0.5 probability of collision-induced catastrophe or crossover. These values are taken from the model used in [2]. Illustration inspired and adapted from [43].

2.3 Elastic MT Model

As with the minimal anchoring model by Tian et al. [42], we make three fundamental assumptions on the processes relating to anchoring and MT bending. First, anchoring proteins are abundant and, on the micrometer scale, have no preferred attachment site along MTs or the membrane. Observations of cortical MTs thus far have not shown evidence otherwise. Although there are potential additional predetermined locations of anchor points along the membrane, these are believed to be sparse [18]. In other words, the majority of anchoring locations are likely not predetermined. Second, MTs seek to minimize the bending energy. It is generally accepted that MTs are relatively stiff filaments

with large persistence lengths [21]. Purely from energy considerations, bending energy likely dominates thermal fluctuations [4]. While factors such as cytoplasmic streaming remain possible additional influences on MT shape [17, 34, 4], these are not considered in this model – as is the case for all models thus far. Third, anchoring proteins clamp the MT in place without providing additional torque to change the MT orientation from its anchored orientation. The possibility of anchoring proteins exerting additional torque or otherwise altering MT shape remains open to exploration, and may be a hypothetical pathway for MT sensing of membrane tension and/or mechanical stress [18]. This is not considered further in this work.

We model the cell as a finite cylinder with MTs as elastic rods growing constrained to the cell's cortex, the cylinder surface. In the simplest case, this is modelled with the CMT being fixed at its originating (minus) end, with the growing (plus) end free. The MT shape is described by that minimizing the curvature energy functional,

$$\mathcal{K}[r(s)] = \int_0^{L(t)} \kappa^2(r(s))\,\mathrm{d}s, \tag{1}$$

where $r(s)$ is the arc-length parametrization of the MT body in $\mathbb{R}^3$, κ the curvature, and $L(t)$ the length of the MT at a given time. The study of such solutions can be found in [5]. Notably, the MT shape depends on the length L of the MT (non-dimensionalized by the cylinder radius) at any moment in time. At a time t, the solution to the variational problem can be found through the parametrization in terms of the tangential angle with respect to the circumferential direction. We denote this parametrization as $\varphi(s;t)$, where s is the arc-length and t is the time which parametrizes the MT length. Therefore, it is necessary to describe the process by which the MTs segment shapes become fixed. This is modelled with anchoring proteins attaching to the body of the MT and fixing MT shapes of segments between anchors. The resultant MT paths are examined in [42]; we provide a brief summary below.

2.3.1 Anchoring Process

Following [42], anchoring is treated as a stochastic process where anchoring proteins attach randomly along the unanchored growing MT tip, with a rate proportional to its length. The constant of proportionality is denoted as k_{on}. This results in a non-dimensional parameter,

$$l_d = \frac{1}{R}\sqrt{\frac{\pi v_g}{2k_{\mathrm{on}}}}, \tag{2}$$

where R is the cylinder radius. This is the mean MT tip length prior to the first anchor attachment, the *mean first anchor length* (not the mean length to the first anchor). For large l_d, the MT tip grows longer and has more opportunity to deflect toward the longitudinal curvature-minimizing direction. l_d therefore controls the influence of membrane curvature on individual MTs. The default

value $k_{\text{on}} = k^*_{\text{on}}$ used here corresponds to the rate constant measured in the model of [1]. Although the aforementioned model is slightly different by including the detachment rate that was found to be comparatively small, this serves as an estimate. Combined with the other default parameters used, we arrive at $l_d = 0.23R$ (4.7μm in dimensional form) being significantly smaller than the smallest of persistence lengths measured *in vivo* [37]. This indicates that bending the curvature influence should dominate thermal fluctuations.

2.4 Order Parameters computed in bands

Because of the competition between edge-induced catastrophe and MT bending in different parts of the domain, we partition the domain into circumferential bands and quantify the angular distributions of CMTs within each band.

We measure order in the 2D domain obtained by unravelling the cylinder surface, ignoring the end caps, and computing the nematic order parameter from [12]

$$S_2 = \frac{\sqrt{\left(\sum_i l_i \cos(2\varphi_i)\right)^2 + \left(\sum_i l_i \sin(2\varphi_i)\right)^2}}{\sum_i l_i}, \tag{3}$$

where l_i are the segment lengths of each MT with corresponding angles φ_i. The angle of $\varphi_i = 0°$ or $\varphi_i = 180°$ corresponding to the circumferential direction. This is derived as the positive eigenvalue of a tensorial order parameter. It measures order where each angle is weighted by the length of their respective segment lengths, and is equivalent to that used in [2]. The order values range from 0 (completely isotropic) to 1 (completely aligned), and is invariant of MT polarity. The dominant direction, Ω, is the orientation of the corresponding eigenvector, given as,

$$\Omega = \arctan\left(\frac{\sum_i l_i \sin(2\varphi_i)}{\sum_i l_i \cos(2\varphi_i) + S_2 \sum_i l_i}\right), \tag{4}$$

for segment lengths and angles $l_i,\ \varphi_i$ as in Eq. 3. The range of Ω is given by the interval $(-90°, 90°)$, reflecting the invariance under polarity.

In order to study how behaviour varies near and far from the end caps, these order parameters are calculated for MT populations along bands of the cylinder, and each band's order parameters are averaged among the respective order parameters over N runs. We use the usual average

$$\overline{S_{2j}} = \frac{1}{N}\sum_{k=1}^{N} S_{2k,j}, \tag{5}$$

where $S_{2k,j}$ denotes the order parameter in the kth simulation, in the jth ban2d. This index notation applies similarly to $\Omega_{k,j}$. However, $\Omega_{k,j}$ is averaged differently; we first take the absolute value, and then average:

$$\overline{|\Omega_j|} = \frac{1}{N}\sum_{k=1}^{N} |\Omega_{k,j}|. \tag{6}$$

This is because directly averaging could result in $\overline{\Omega}_j = \frac{1}{2}(-45° + 45°) = 0$, which would be misleading because both angles are equally diagonal.

2.5 Simulation Method: Individual MTs

We independently implement the event-driven simulation method outlined in [43] and generalize to the case of curved MTs. As the length of a MT changes, it "sweeps" through various mechanical equilibria (energy minimizers), driving a lateral movement. For illustration purposes, we refer to the top of Fig. 1 where this sweeping is visible as the length increases. All models thus far approximate MTs as following paths without sweeping; we term this the "fixed path approximation". A model with sweeping MTs is computational challenging; we therefore also make use of a fixed path approximation. Coupling this with the anchoring process allows us to capture MT deflection with control on the various errors introduced. We provide a brief explanation below; a thorough analysis of this approximation is the subject of separate manuscript in preparation.

For this section, we assume a non-dimensionalization where length is in units of the cylinder radius R and time is in units of time required for polymerization to grow a MT to a length of R. The deflection of MTs only depends on MT lengths relative to the radius, and the speed is such that a MT will grow to a length t after t time units. We parametrize the MT path with its tangential angle as a function of arc-length and time, $\varphi(s;t)$, resulting from the variational problem. Our fixed path approximation comes from a spatio-temporal approximation on the MT shapes. We describe it in Sec 2.5.1 and 2.5.2, with an accompanying illustrative summary in Fig. 3.

2.5.1 Step 1: Temporal Approximation

Suppose we seek to simulate a MT of length L. We first approximate the MTs as following curves that are fixed in time. Although MT segments eventually become fixed in shape due to the anchoring model, their shapes nonetheless sweep as they grow prior to being fixed (as described by the solutions to Eq. 1). Referring to the visual in Fig. 1, the growing MT sweeps through the various green curves before finally reaching the the longest green curve. We approximate the MT growing along the longest green curve rather than simulating each smaller curve as time progresses. Mathematically, the approximation made is,

$$\varphi(s;t) \approx \varphi(s;T), \quad T >> L. \tag{7}$$

Here, T is a time after all anchors have fixed the MT shape up to length L. This is illustrated in Step 1 of Fig. 3. In this case, we are only interested in the MT for times before the first anchor, so $T > t_4$ is the time after the first anchor has fixed the shape. Although visibly inaccurate in the exaggerated case of Fig. 3, this approximation can be justified in the case where l_d is sufficiently small. In such cases, MTs do not sweep significantly on each individual segment. This approximation only applies to the freely growing tip. Furthermore, in the case of small l_d, the segments of barrier MTs will likely be already anchored

prior to collisions, meaning the inaccuracies in collision geometry caused by this approximation are reduced.

2.5.2 Step 2: Spatial Approximation

We now construct a numerical approximation to the curve $\varphi(s;T)$. First, we solve the variational problem for φ numerically using a boundary value problem solver with a fine grid size δs. It remains to coarsen the spatial discretization of the curve for the purposes of resolving collisions in a computationally efficient manner. This is because MTs will be approximated as line segments, and many segments result in increased calculations for collision detection. We set a coarse discretization $\Delta\tilde{s}$ that provides an upper bound on the segment lengths used in the simulation. Suppose there is an anchored segment with anchor points $s_1 < s_2$. Then, the discretization represents the actual path $\varphi(s;T)|_{s\in[s_1,s_2]}$ using piecewise linear segments of lengths Δs_i with corresponding subdivision points $\{s'_i\}$ on the interval $[s_1, s_2]$. If there are n segments, these segment lengths are given by,

$$\Delta s_i = \begin{cases} \Delta\tilde{s}, & i \neq n \\ \min\left(\Delta\tilde{s}, s'_{i+1} - s'_i\right), & i = n. \end{cases} \tag{8}$$

In words, all segments have $\Delta s_i = \Delta\tilde{s}$ except for the last one where it is possibly a remainder that is less than $\Delta\tilde{s}$. We choose to write the coarse segment angles $\tilde{\varphi}$ as the average,

$$\tilde{\varphi}(s)|_{s\in[s'_i,s'_{i+1}]} = \frac{1}{s'_{i+1} - s'_i} \int_{s'_i}^{s'_{i+1}} \varphi(x,T)\,\mathrm{d}x. \tag{9}$$

This coarsening method is chosen such that the length of the MT is preserved, different from e.g. an interpolation method. A result of this is that the coarsened linear approximation is not necessarily composed of secants or tangents on the original curve, as illustrated in Step 2 of Fig. 3. There is no longer a dependence on time t since the MT now has a fixed path up to the length of interest. With these values for MT angles, we represent MTs by mapping to cylindrical coordinates.

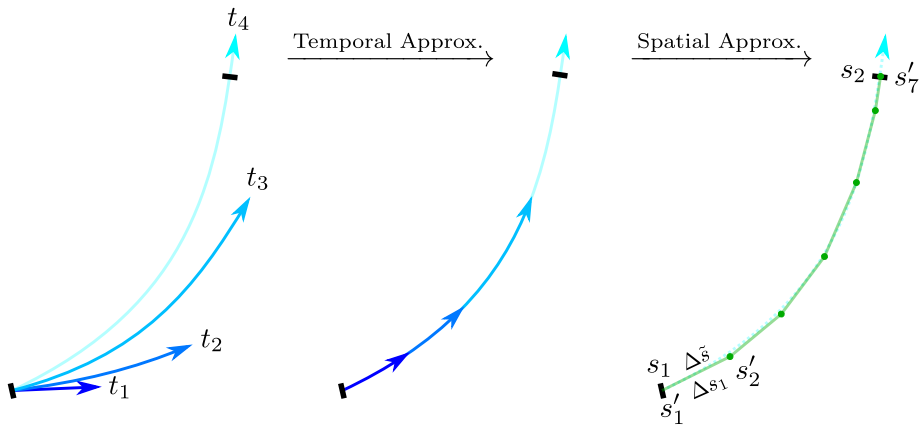

Figure 3: Approximating MT paths as fixed paths. Temporal approximation: the MT body (shades of blue on the leftmost curves) sweeps as it grows. For times before the anchor time t_4, the MT is approximated as tracing the future anchored segment (light blue corresponding to t_4) in Eq. 7. Spatial approximation: the path is spatially discretized (green polygonal curve) as in Eq. 9. Labelled are the anchor points s_i, discretized subdivision points s_i', upper-bound segment length $\Delta\tilde{s}$, and implemented segment lengths Δs_i. Note the spatial approximation is not simply an interpolation, see Sec 2.5.2.

In the present work, the fine grid solution is solved with $\delta s = 10^{-2}$ using SciPy's `ode.solve_bvp` with a tolerance of 10^{-4}. The tolerance controls the accuracy of the solution, whereas the fine grid determines the desired points of the solution to solve for. Then, the coarse spacing is set as a function of initial MT angle φ_0. Without loss of generality, we restrict to $\varphi_0 \in [0°, 90°]$,

$$\Delta\tilde{s} = \begin{cases} 0.05, & 0° \leq \varphi_0 \leq 35.5°, \\ 0.1, & 35.5° < \varphi_0 \leq 63.0° \\ 0.2, & 63.0° < \varphi_0 \leq 90°. \end{cases} \tag{10}$$

The reasoning for the piecewise values is because solutions with initial angles close to 90° do not vary much. Therefore, we do not need $\Delta\tilde{s}$ to be small. The choice of where to divide the piecewise function is otherwise arbitrary.

We find this choice to give an average approximation error to be less than 1° on individual MT shapes. We leave further error analysis to a manuscript in preparation.

2.6 Simulation Method: Interacting MTs

Conceptually, the simulation algorithm follows that described in [43]. There are deterministic and stochastic events. The deterministic events are collisions between the line segments representing MTs whose times are calculated given the current state of the system. The stochastic events are sampled using a kinetic

Monte Carlo (Gillespie) algorithm. All events are sorted by their times, and the earliest is executed. Upon execution, the appropriate changes in simulation state are made and new events are calculated and sampled. This is repeated.

In addition to the usual interactions described in Sec. 2.2, the introduction of MT bending requires new decisions on MT dynamics within bundles. As with [43], bundles are computationally represented as occupying the same curve such that collisions with bundles are equivalent to collision with a single MT. We identify bundles as a partition on the set of MTs, making "bundling" an equivalence relation on this set. Being able to distinguish between bundles allows us to resolve collisions between entrained MT bundles, as illustrated in Fig. 4. We keep track of the "sidedness" of entrained MTs, such that we can resolve the collision illustrated in Fig. 4b). In this sense, our simulation captures todoelements of the simulation methods in [2, 3, 30] where the non-zero spacing of entrained MTs is explicitly represented. This differs from the choice made in [43] and other studies based on the methods described therein [44, 12]. Our method also allows for ignoring MT "sidedness", and we note that this generally gives similar results albeit with higher ordering S_2. The question of which choice is most appropriate remains open; this relies on more precise measurements on the nature in which MTs bundle.

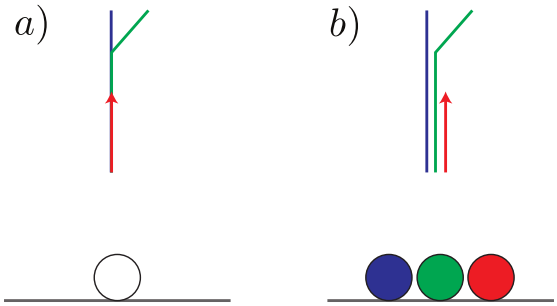

Figure 4: Representation of bundles within the simulation. a) Entrained MTs are represented as occupying the same curve. b) illustrates an alternative way of representing bundles where entrained MTs do not occupy the same curve. In the case of b), the leftmost red MT is able to resolve the collision with the entrained centre green MT. Although our simulation uses representation a), we keep track of the "sidedness" of entrained MTs, maintaining one of the benefits of b).

Lastly, there are new events associated with the "freeing" of bundled MTs, whereby an entrained MT is no longer entrained. Once this occurs, the liberated MT is allowed to deflect, as illustrated in Fig. 5.

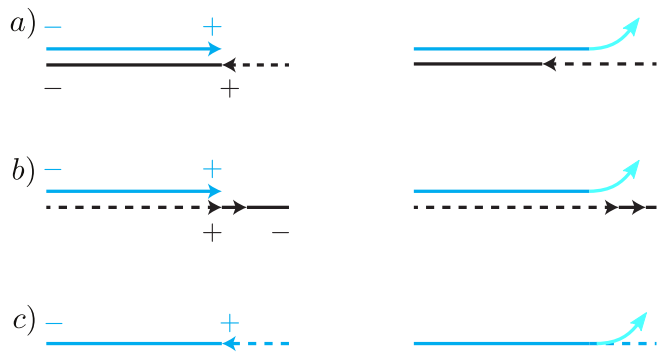

Figure 5: Illustration of how a MT of interest (blue) may branch out of its entrainment (black) through: a) blue MT catching up to the shrinking black MT, b) the black MT shrinking faster than the blue MT is growing, c) the blue MT is shrinking but rescued. In all these cases, the blue MT tip is freed and there is a new path (light blue) because there is a new sequence of anchors starting from its growing tip and hence a potentially different deflection. This is as opposed to the blue MT re-tracing the path it was previously was on.

2.7 A Model For Angle-Dependent Catastrophe: Cellulose Feedback

Previous experimental work has observed changes in CMT orientation after perturbing the cellulose microfibril ordering by treatment with a cellulose synthesis inhibitor [20]. In particular, there was a statistically significant increase in deviation of CMT transverse orientation after the microfibrils lost order. Subsequent studies have shown that mutations affecting cellulose production and quality can alter CMT orientation [9, 26]. This motivates the following model.

To investigate a hypothetical mechanism to compete with the longitudinal bias caused by MT bending, we propose an angle-dependent catastrophe rate that increases catastrophe along the longitudinal direction. For example, this angle dependence could be due to the hindering of CMT polymerization in the presence of transversely oriented cellulose microfibrils.

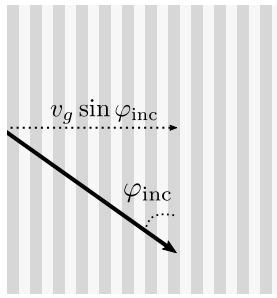

Figure 6: Illustration of a model for cellulose feedback. While a MT grows at speed v_g with angle of incidence φ_{inc}, its tip encounters ridges of transversely oriented cellulose microfibrils. The rate at which it encounters the ridges is proportional to its horizontal speed $v_g \sin \varphi_{\text{inc}}$.

In this work, we follow the commonly used model of MT dynamic instability in which the switch between states is a Poisson process. In particular, the probability of a polymerizing MT undergoing catastrophe in a small time span of Δt given by $r_c \Delta t$ where r_c is the catastrophe rate. Potentially, transversely deposited cellulose microfibrils form ridges along inner side of the membrane, hindering the polymerization process of a MT growing with angle of incidence φ_{inc} with respect to the cellulose microfibrils. Given the high turgor pressure of plant cells, protoplast tension should enable CMTs to sense the cell wall textural landscape. As a first approximation, we assume the probability of this catastrophe factor is proportional, with constant of proportionality λ, to the number of ridges $m(\Delta t)$ encountered in a time interval Δt. To determine the number of ridges encountered, we define the ridge density ρ, measuring the number of transverse cellulose microfibrils per unit vertical distance. This gives the number of ridges encountered as proportional to vertical distance travelled Δz multiplied by this density. In all, the probability of a MT polymerizing at speed v_g undergoing catastrophe is give by the sum of its intrinsic instability $(r_0 \Delta t)$ and the cellulose feedback factor,

$$\begin{aligned} r_c \Delta t = &= r_0 \Delta t + \lambda m(\Delta t) \\ &= r_0 \Delta t + \lambda \rho \Delta z \\ &= r_0 \Delta t + \lambda \rho v_g \sin \varphi_{\text{inc}} \Delta t \\ &= r_0 \left(1 + \varepsilon \sin \varphi_{\text{inc}}\right) \Delta t, \quad \varepsilon = \frac{\lambda \rho v_g}{r_0}. \end{aligned}$$

As a result, we can define the angle-dependent catastrophe rate,

$$r_c(\varphi_{\text{inc}}) = r_0 \left(1 + \varepsilon \sin \varphi_{\text{inc}}\right). \tag{11}$$

We note that φ in this case refers to the angle of incidence at the MT tip.

There remains a question of how to related this to the observed catastrophe rate r_{obs}. This previously measured rate does not take into account the possibility of angle dependence, and we seek to normalize r_c with respect to r_{obs}. Given

$N(t)$ growing MTs at a particular time, the total catastrophe rate is $N(t)r_{\text{obs}}$. Introducing a MT population density $n(\varphi_{\text{inc}}, t)$ where $\int_0^{\pi/2} n(\varphi, t)\,\mathrm{d}\varphi = N(t)$, we set the equality,

$$N(t)r_{\text{obs}} = \int_0^{\pi/2} n(\varphi, t) r_c(\varphi)\,\mathrm{d}\varphi \tag{12}$$

$$= r_0 \int_0^{\pi/2} n(\varphi, t)\,(1 + \varepsilon \sin\varphi)\;\mathrm{d}\varphi \tag{13}$$

$$= r_0 N(t) + r_0 \varepsilon \int_0^{\pi/2} n(\varphi, t) \sin\varphi\,\mathrm{d}\varphi \tag{14}$$

$$\implies r_0 = \frac{N(t) r_{\text{obs}}}{N(t) + \varepsilon \int_0^{\pi/2} n(\varphi, t) \sin\varphi\,\mathrm{d}\varphi} \tag{15}$$

Since we do not have an analytic expression for $n(\varphi, t)$, this is challenging to solve exactly. However, our goal is not to solve this exactly. It is not necessarily true this relation holds for all times. By introducing this new model, we are assuming that r_{obs} is no longer sufficient in describing the catastrophe rate and that the true process is angle-dependent. Instead, we normalize to the instance in time in which r_{obs} is measured. Under the assumption that this was measured at a time the distribution was uniform, $n(\varphi_{\text{inc}}, t) = N(t)/\frac{\pi}{2}$, we can solve Eq. 15 to get,

$$r_0 = \frac{r_{\text{obs}}}{1 + \frac{2\varepsilon}{\pi}}. \tag{16}$$

In all, our model gives the angle-dependant catastrophe rate as,

$$r_c(\varphi_{\text{inc}}) = \left(\frac{r_{\text{obs}}}{1 + \frac{2\varepsilon}{\pi}}\right)(1 + \varepsilon \sin\varphi_{\text{inc}})\,. \tag{17}$$

3 Results

To understand the interplay between the transverse-biasing influence of edge-induced catastrophe and the longitudinal-biasing influence of bending energy minimization on CMT organization, simulations were first run with the default parameters in Table 1 while the anchoring rate k_{on} and hence the degree of MT deflection was varied. Afterwards, we investigated the impact of cell geometry by repeating this on a shorter cell. Finally, we tested whether a hypothetical angle-dependent catastrophe rate can robustly generate transverse arrays despite the presence of MT deflection.

3.1 Anchoring Provides Control For Global Longitudinal Ordering; Edge-Induced Catastrophe Is a Comparatively Weak Influence

To investigate the role of anchoring in controlling MT bending, we varied the anchoring rate k_{on} by using multiples of the experimentally estimated values k_{on}^* or taking the geodesic (straight line) limit on a long cell. This is shown in Fig. 7. We found the longitudinal influence to be strong for the experimentally measured $k_{\text{on}} = k_{\text{on}}^*$. Although there was strong transverse order (low $|\Omega|$ and high S_2) of CMTs close to the edges due to edge-induced catastrophe, the longitudinal bending quickly became dominant (high $|\Omega|$ and moderate S_2) toward the middle of the cell. In the transition from the edge toward the middle, this competition between the two influences was seen by the lower S_2 observed in the second and third bands from the edges. By increasing k_{on}, thereby decreasing the deflection, this longitudinal influence was indeed mitigated. Indeed, the geodesic limit was approached as k_{on} became larger. This is consistent with our previous hypothesis [42] of anchoring being a potential mechanism for controlling array orientation. Whereas the experimentally estimated rate resulted in strong longitudinal ordering, roughly an order of magnitude increase gave ordering similar to the geodesic case. However, the ordering of the geodesic case deserved particular attention.

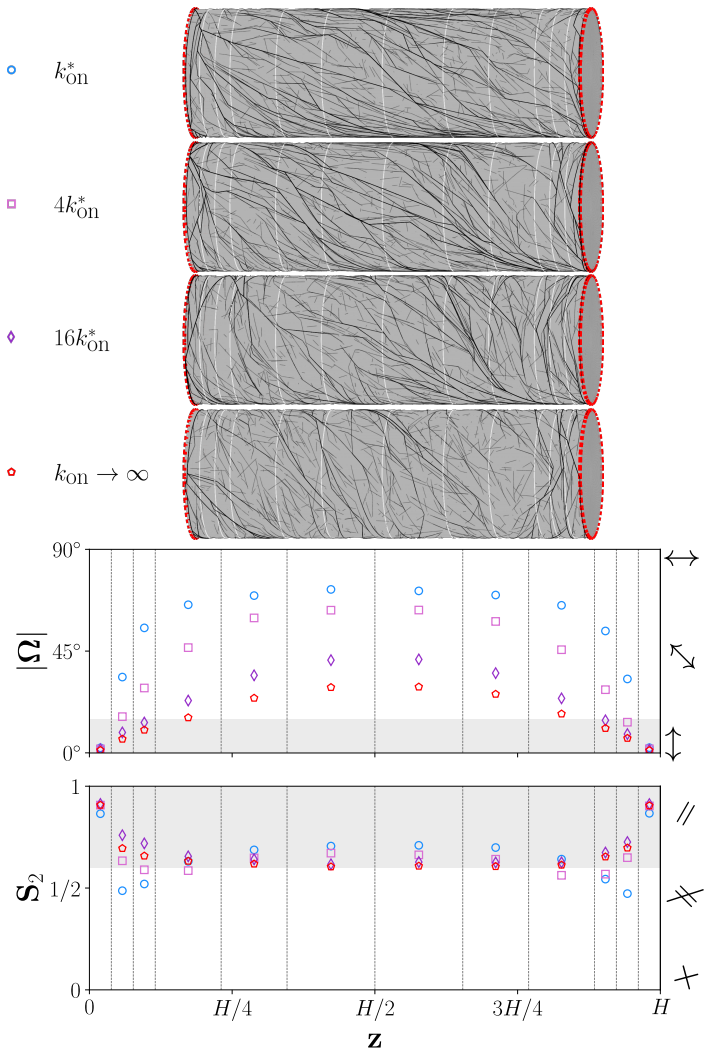

Figure 7: Ordering of cortical microtubule arrays plotted for various values of the anchoring rate k_{on}, with all other parameters set as default in Table 1 on a long cell. Top: visualization of individual simulations along cylindrical cells. Microtubules are not visualized underneath the cylinder surface facing the reader. Red dotted lines indicate catastrophe-inducing edges and white lines indicating the domain partitions. Anchoring rates used are multiples of the experimentally estimated value k^*_{on}, or taking the infinite limit corresponding to geodesics (straight-line MTs). The corresponding values are labelled on the left of each cylinder. Bottom: averages of order parameters $|\Omega|$ (dominant angle) and S_2 (order) plotted along the respective bands of the cylindrical domain set with the long axis horizontal (z). Shaded regions provide a qualitative estimate of expected order parameters for transverse microtubule arrays: $S_2 \geq 0.6$ [41] and $|\Omega| \leq 15°$. Average order parameters are calculated by considering the angular distribution for microtubules within each band of the domain after 10h simulation time, and then averaging over 100 simulations. Standard error of the mean (not shown) is approximately the size of each indicator.

3.2 Anchoring And Edge-Induced Catastrophe Cannot Reliably Recover Transverse Ordering In A Large Domain

Even after taking the limit to geodesics, thereby eliminating the bending influence, edge-induced catastrophe could not reliably recover a globally transverse array for the long cell geometry (see red pentagon data points in the $|\Omega|$ plot of Fig 7). This showed that edge-induced catastrophe had a weak effect on larger cells in the best possible case. We further investigated the local order parameters in the geodesic case by comparing the spread of the distributions with that of the MT bending case in Fig. 8. Toward the middle of the domain for the geodesic case, we found S_2 to be moderate with a large variance around $|\Omega|$. This indicates that, farther from the edges, there is no strong preference for the orientation taken among different cells; organization arises randomly due to MT-MT interactions with a weak tendency toward the transverse direction. In the case of default anchoring, the longitudinal ordering was slightly more robust. This was seen by the smaller spread in order parameters away from the edges, and faster convergence in Figs. S11 & S12. The observation of weak transverse ordering due to edge-induced catastrophe within larger domains, even in the geodesic limit, is consistent with previous observations of weaker order parameters [43, 27]. With the exception of a recent study done in parallel by Li et al. [27], previous studies to our knowledge have focused on determining the order parameters on a global scale, ignoring the possible effects of over/underestimating order parameters due to local variations such as edge-induced catastrophes.

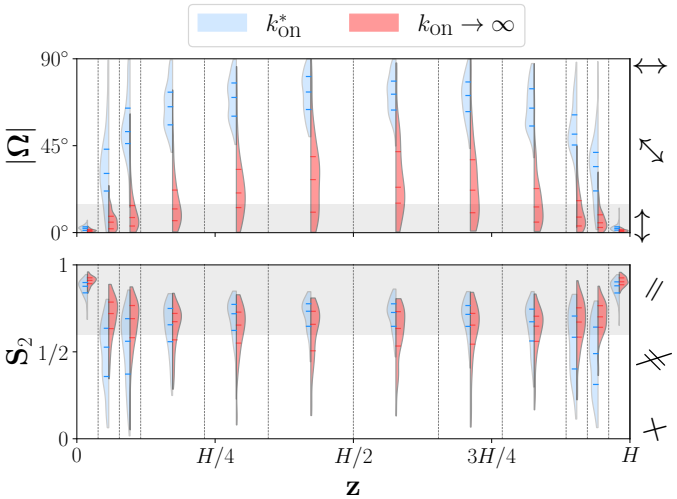

Figure 8: A closer examination of the ordering statistics in Fig. 7, using the same data. Violin plots are used to provide a qualitative visualization of order parameter distributions for chosen anchoring rates. Lines within each violin half-plot indicate the first, second, and third quartiles.

3.3 Within Short Cells, Edge-Induced Catastrophe Produces More Transverse Arrays

As shown above, transverse MTs appear in boundary layers localized to the edges. We investigated whether a globally transverse array can form in the presence of MT bending by bringing the boundary layers closer i.e. shortening the cell. Indeed, as seen in Fig. 9, we arrived at CMT arrays more consistent with *in vivo* observations. However, we note that the ordering near the middle was slightly oblique. This raises the question of how to define the CMT as being "sufficiently transverse". Although there have been measurements of angular distributions in various experiments (e.g. [3, 20, 28]), there does not appear to be sufficient data for a direct quantitative comparison of order parameters as measured *in vivo* to a statistically meaningful extent.

Taking the geodesic limit, we found strongly transverse arrays. This is consistent with previous modelling studies that focused on domains with a similar aspect ratio and/or prism-shaped cells with flat faces where MTs travel along straight lines [3, 28]. *In vivo*, this would correspond to recently divided cells entering elongation. It remains a possibility that the transverse arrays found in longer elongating cells are built on the existing array from when the cells where shorter. However, depolymerization experiments on already long elongating cells nonetheless recovered the transverse ordering from an (assumed) initially empty state. This is the same context in which our simulation takes place. We therefore examined a potential process to recover transverse arrays in the next section.

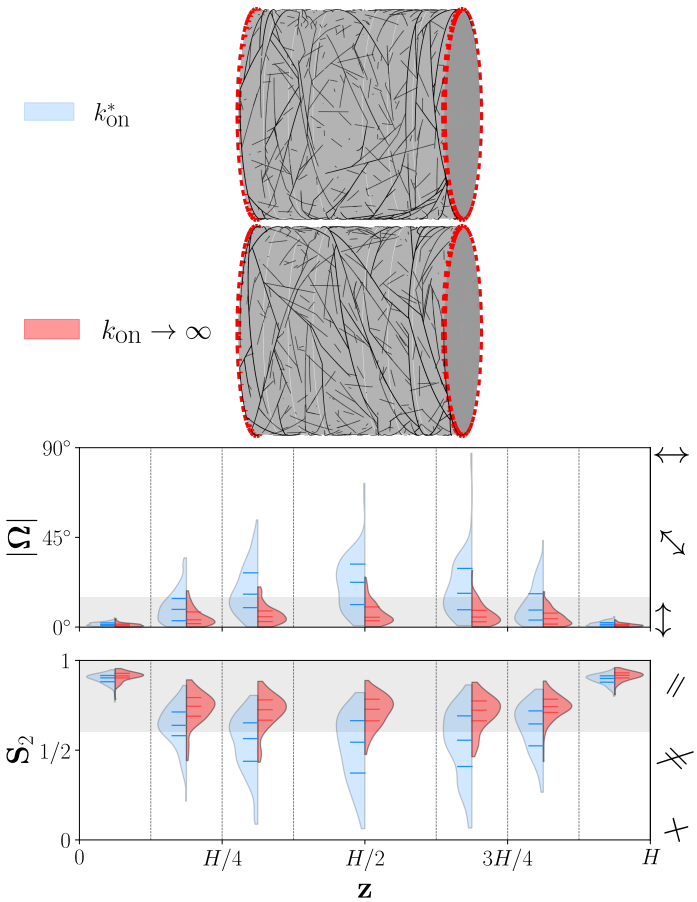

Figure 9: Ordering of the cortical microtubule array plotted for two values of the anchoring rate k_{on}, with all other parameters set as default in Table 1 on a short cell. Top: visualization of individual simulations along Anchoring rates used are multiples of the experimentally estimated value k^*_{on}, or taking the infinite limit corresponding to geodesics (straight lines). Bottom: violin plots of the order parameters in each band. .

3.4 In Long Cells, Moderate Angle-Dependent Catastrophe Allows For Transverse Ordering Despite Longitudinal Bending Bias

We have shown that in the geodesic limit in long cells, global transverse ordering fails. With the addition of the longitudinal MT bending, even short cells failed to order transversely far from the catastrophe-inducing edges. The question remained as to how the transverse CMT ordering process can overcome the theoretical bending influence. A possible mechanism is the favouring of transverse MTs through an orientation-dependent regulation of dynamic instability – an

idea that has been proposed as early as 1989 by Wasteneys and Williamson [49]. This could equivalently occur by antagonizing MTs of less desirable orientations through destabilization. We investigated a model in which MTs exhibit angle-dependent catastrophe as a consequence of encountering the transverse texture (speed bumps) delineated by cellulose microfibrils.

We introduced angle-dependent catastrophe as described in Sec. 2.7), with the intensity of angle dependence relative to intrinsic MT instability parametrized by ε in Eq. 11. In Fig. 10, we found this global biasing mechanism could overcome the longitudinal influence ($k_{\text{on}} = k^*_{\text{on}}$) and create transverse arrays even with a moderate value of $\varepsilon = 0.8$. This corresponds to not quite doubling the catastrophe rate of longitudinal MTs relative to that of transverse MTs. This mechanism is robust in the sense that there is less spread in both $|\Omega|$ and S_2 (Fig. 14) about the values of transverse ordering. We further observed that, for $\varepsilon = 0.8$, the convergence time of the mean S_2 parameter was quicker than the geodesic case. It was comparable to the speed of ordering due to edge-induced ordering in the bands closest to the edges. This is consistent with the results in a recent study using a different computational method in the context of a three-state MT model using arabidopsis dynamic instability parameters[27]. There it was shown that the modification of catastrophe rates has the most potent effect on both strength and speed of ordering. This could be an additional factor needed to speed the ordering in simulations to times more accurate to those observed *in vivo*.

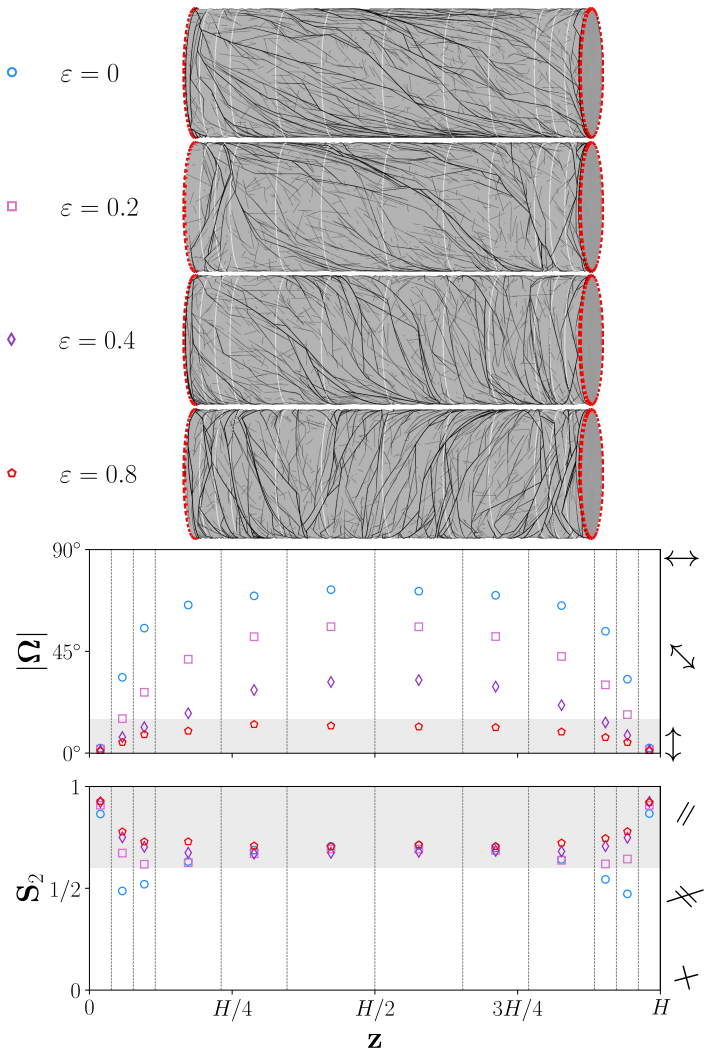

Figure 10: Ordering of the cortical microtubule array plotted for various values of angle-dependent catastrophe strength ε, with all other parameters set as default in Table 1 on a long cell. Top: visualization of individual simulations. Bottom: averages of the order parameters in each respective band. Order parameters are calculated as in Fig. 7 except not all were 10h simulations since larger ε simulations organize and saturate in MTs quickly (see Supplementary Figs. 12&13), slowing the simulation down.

4 Discussion

As CMT models become generalized to complicated geometries, one must consider whether previous assumptions remain applicable. In particular, the ap-

proximation of MTs travelling along straight lines (geodesics) has remained unchallenged. We present the first model to generalize the previous MT-MT interaction modelling formalisms by explicitly modelling the mechanical bending of MTs. This is done on a cylindrical cell, using biologically relevant parameters and dimensions. We have shown such bending mechanics should be significant, especially in long cells. The tendency of individual MTs to minimize bending energy translates to a strong influence on the collective array organization *in silico*. As a result, the array orients to the longitudinal direction. The presence of edge-induced catastrophe – a generally accepted mechanism for transverse orienting of CMT arrays – is no longer sufficient in this geometry. Modulating the degree of bending via membrane-MT protein anchoring rate offers some control over this deviation from transverse arrays. In the case of short cells, it remains possible for edge-induced catastrophe to be sufficient for transverse arrays, consistent with observations of the short cells in the root division zone of *clasp-1* mutants [3]. That shorter cells may still obtain transverse arrays provides an interesting hypothesis: is it possible that edge-induced catastrophe produces the initial transverse array to kick-start the anisotropic expansion, after which there is a positive feedback loop from cellulose and/or stress/tension in the membrane? Observations in *Nitella* internodal cells indicate a correlation between the strain rate of elongating internodal cells and the prevalence of transverse MTs [33]. A model including an expanding domain may be of interest for future work.

Nonetheless, the observation of transverse CMT arrays recovering after depolymerization in longer cells [49, 28] suggests the presence of additional processes to overcome the expected longitudinal bias. The necessity of additional processes is further illustrated by depolymerization experiments in *Nitella* [49]. In such a system, the domain is extremely large (5-15 mm in length, unpublished data [49]) compared to the average MT length ($10\,\mu$m). Although bending effects are expected to be weaker due to the larger radii of *Nitella* cells, one may expect edge-induced catastrophe to be extremely weak simply due to the domain size. Furthermore, CMT self-organization on this scale solely due to MT-MT interactions may not be as robust. Yet, transverse CMTs manage to consistently recover. Potentially, such additional processes could involve mechanical forces shifting individual MTs toward the transverse direction. In such cases, MTs may no longer minimize bending energy and instead satisfy and alternative energy/force formulation. No observations thus far support the existence of such a process. Instead, we find this work to point toward an idea alluded to by Wasteneys and Williamson [49]: the existence of a "non-kinematic" process favouring transverse MTs through an angle-dependent modification of dynamic instability. This is a reasonable hypothesis given the observed geometric effects of membrane shape and plethora of microtubule-associated proteins that are implicated to affect dynamic stability [3, 24, 50, 29, 32]. We have shown that a moderate angle-dependence, such as through cellulose feedback, could result in robust transverse ordering despite the longitudinal bias induced by MT bending. The existence of such a dependence may be tested by measuring the catastrophe rates of MTs in various angles with or without the presence of transversely

oriented microfibrils. In addition, there is a long-standing hypothesis of MT mechanosensing [19, 18]. Currently, such mechanism(s) are largely unknown, thus difficult to model. We note that the cellulose hypothesis and the idea of MTs being sensors of mechanical stress or tension are not mutually exclusive. If membrane tension affects MT stability in a cylindrical cell, such a mechanism may introduce angle-dependent catastrophe similar to what we introduced here. Indeed, such ideas were tested in a simple context of a flat simulation domain by Li et al. [27] by assuming a hypothetical linear relation between stress and MT dynamic instability parameters including and not limited to catastrophe. Their angle-dependent expression is mathematically similar to our model. Although their method differed in model parameters (arabidopsis vs. tobacco) and computational implementation (differing in algorithm and representation of MTs), it has nonetheless shown similar effects on CMT organization. This indicates a consistent need for such processes across plant models.

Our study focused on a minimal set up without severing or alternative modes of nucleation, which are known to impact MT organization *in silico* and *in vivo* [2, 15, 12, 11]. *In vivo*, severing has been implicated in assisting the process of CMT reorientation in response to blue light [28] and stress in the cell wall [45]. *In silico*, severing has been shown to increase or decrease the ordering of CMT arrays depending on the location of severing and subsequent behaviour of severed MTs. However, models of severing thus far have not shown a biasing effect on the overall CMT orientation. Thus, we do not expect existing severing models to significantly impact the orientation of CMTs observed in this work. Recent work on modelling a more realistic nucleation process [36] indicates that including such a nucleation process may decrease the sensitivity of CMT arrays to biasing cues and promote transverse arrays in the case of long cells with small radii. In the geometry studied presently, we suspect this would not significantly affect the longitudinal influence. We hypothesize that such locally density-dependent nucleation favours transverse arrays due to the smaller average-MT-length-to-circumference ratio. When this ratio is small, it is easier for transverse MTs to wrap around and approach their own minus ends – thereby creating denser arrays in this orientation. However, our work assumes a larger radius in comparison to average MT length. Indeed, from observations of CMTs in relatively large *Nitella* cells, it has been hypothesized that MT-dependent nucleation creates disorder in the CMT array [48]. Future work is needed to test the interplay between increased longitudinal bias (from MT bending) and promotion of transverse arrays (from local density-dependent nucleation) due to decreasing the radius. Another degree of freedom unexplored in this work is the effect of changing the dynamic instability parameters aside from angle-dependence, either as constants or time-varying. By decreasing the average MT length, which is governed by the activity of catastrophe factors [14] it might be possible to mitigate the deflection of individual MTs. Regardless, by keeping with a commonly used default parameter set [43, 12], our work serves to indicate the importance of this bending influence.

All MT models thus far, including this model, have a common limitation: MTs are approximated as polymerizing along "fixed paths" where the MT body

does not sweep laterally in response to mechanical forces. The fixed path approximation can be made to accurately model mechanical influences by considering the anchoring process; the accuracy relies on the average anchor length parameter l_d being small. Although this excludes the study of contexts where such movement is extreme, this model provides an approximation, a perturbation, of the fixed path model into a model of laterally dynamic MTs. The coupling of MT mechanics and anchoring provides a first step toward a more general model that has the potential to capture aspects such as the dynamic reorientation of CMT arrays. As applied to the context of bending mechanics, the observation of a "default" tendency for MTs to orient in the longitudinal direction may be a factor in the transition from transverse to longitudinal arrays. This is a tempting hypothesis since this bending is simply due to the physics of MT polymers; the cells would not need to expend additional resources for this to occur. Furthermore, it may be possible to incorporate the mechanics induced by cytoplasmic streaming, hypothesized to also play a role in reorientation [17, 34].

5 Supplementary Material

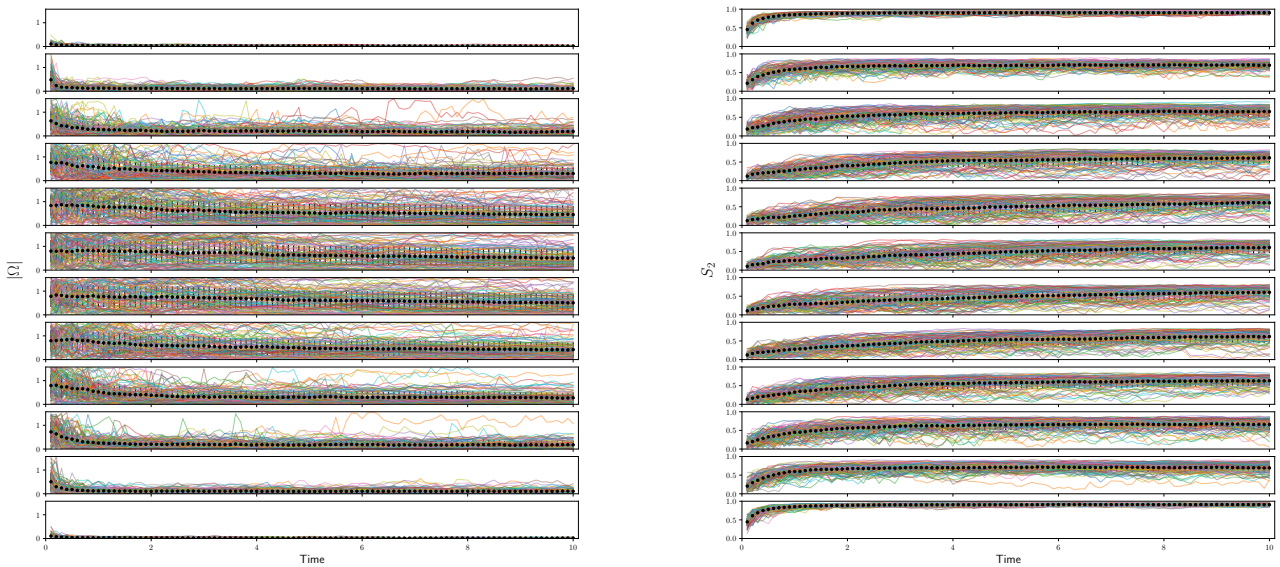

Figure 11: Order parameters over time for cell long, geodesic case. Each plot is split into the respective bands of the cylinder.

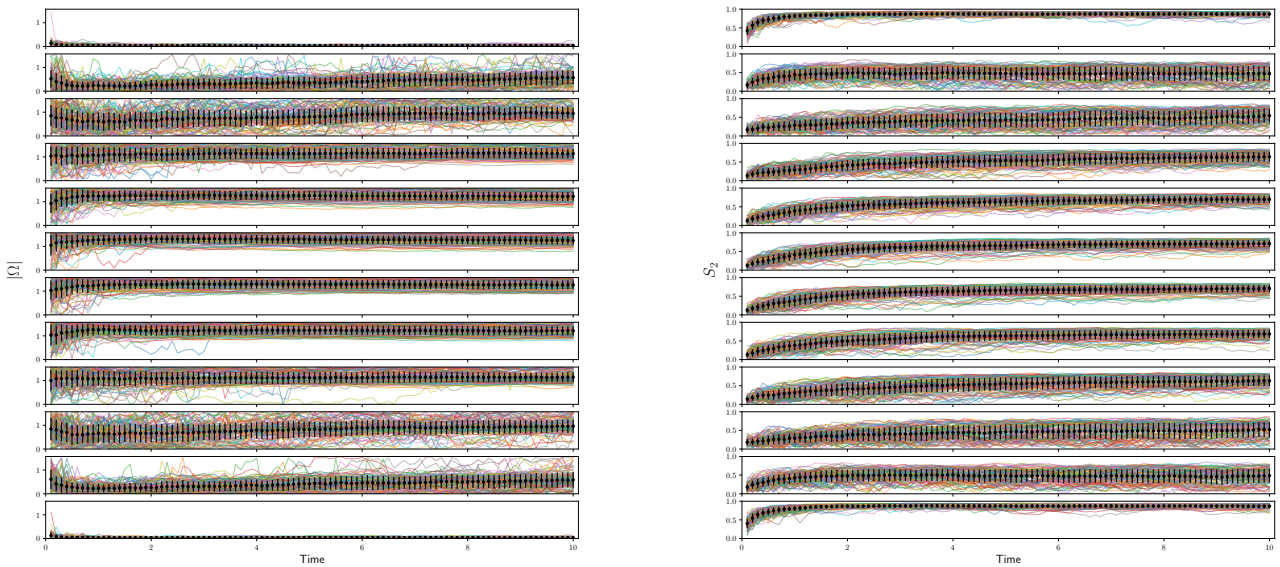

Figure 12: Order parameters over time for long cell with k_{on}^{*}

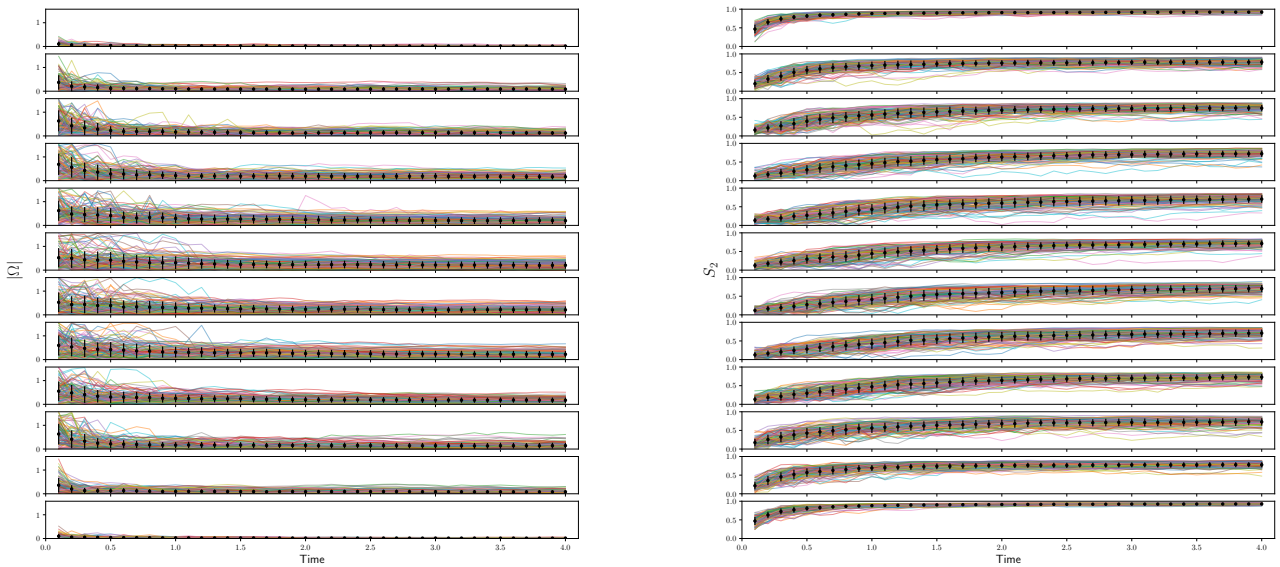

Figure 13: Order parameters over time for long cell with $\varepsilon = 0.8$

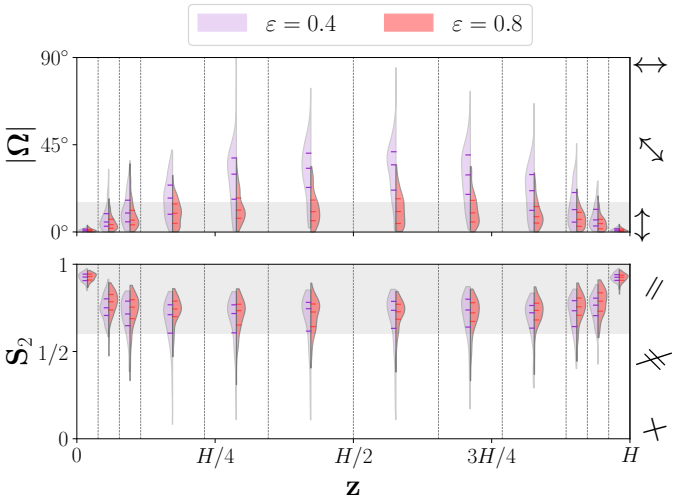

Figure 14: A closer examination of the ordering statistics in Fig. 10, using the same data.

References

[1] Jun F. Allard, J. Christian Ambrose, Geoffrey O. Wasteneys, and Eric N. Cytrynbaum. A mechanochemical model explains interactions between cortical microtubules in plants. *Biophysical Journal*, 99(4):1082–1090, 2010.

[2] Jun F. Allard, Geoffrey O. Wasteneys, and Eric N. Cytrynbaum. Mechanisms of self-organization of cortical microtubules in plants revealed by computational simulations. *Mol. Biol. Cell*, 21(2):278–286, 2010.

[3] Chris Ambrose, Jun F. Allard, Eric N. Cytrynbaum, and Geoffrey O. Wasteneys. A clasp-modulated cell edge barrier mechanism drives cell-wide cortical microtubule organization in arabidopsis. *Nature Communications*, 2(1), 2011.

[4] J. Christian Ambrose and Geoffrey O. Wasteneys. CLASP modulates microtubule-cortex interaction during self-organization of acentrosomal microtubules. *Molecular Biology of the Cell*, 19(11):4730–4737, 2008. PMID: 18716054.

[5] Sven Bachmann, Richard Froese, and Eric N. Cytrynbaum. A buckling instability and its influence on microtubule orientation in plant cells. *SIAM Journal on Applied Mathematics*, 79(5):2132–2149, 2019.

[6] Tobias I. Baskin. Anisotropic expansion of the plant cell wall. *Annual Review of Cell and Developmental Biology*, 21(1):203–222, 2005.

[7] Bandan Chakrabortty, Ikram Blilou, Ben Scheres, and Bela M. Mulder. A computational framework for cortical microtubule dynamics in realistically shaped plant cells. *PLOS Computational Biology*, 14(2):1–26, 02 2018.

[8] Jordi Chan, Adrian Sambade, Grant Calder, and Clive Lloyd. Arabidopsis cortical microtubules are initiated along, as well as branching from, existing microtubules. *The Plant Cell*, 21(8):2298–2306, Aug 2009.

[9] Zhaoqing Chu, Hao Chen, Yiyue Zhang, Zhonghui Zhang, Nouyan Zheng, Bojiao Yin, Hongyan Yan, Lei Zhu, Xiangyu Zhao, Ming Yuan, Xiansheng Zhang, and Qi Xie. Knockout of the *AtCESA2* gene affects microtubule orientation and causes abnormal cell expansion in arabidopsis. *Plant Physiology*, 143(1):213–224, 11 2006.

[10] Leia Colin, Antoine Chevallier, Satoru Tsugawa, Florian Gacon, Christophe Godin, Virgile Viasnoff, Timothy E. Saunders, and Olivier Hamant. Cortical tension overrides geometrical cues to orient microtubules in confined protoplasts. *Proceedings of the National Academy of Sciences*, 117(51):32731–32738, 2020.

[11] Eva E. Deinum, Simon H. Tindemans, Jelmer J. Lindeboom, and Bela M. Mulder. How selective severing by katanin promotes order in the plant cortical microtubule array. *Proceedings of the National Academy of Sciences*, 114(27):6942–6947, Jun 2017.

[12] Eva E Deinum, Simon H Tindemans, and Bela M Mulder. Taking directions: The role of microtubule-bound nucleation in the self-organization of the plant cortical array. *Physical Biology*, 8(5):056002, Jul 2011.

[13] Ram Dixit and Richard Cyr. Encounters between dynamic cortical microtubules promote ordering of the cortical array through angle-dependent modifications of microtubule behavior. *The Plant Cell*, 16(12):3274–3284, 2004.

[14] Ryan Christopher Eng and Geoffrey O. Wasteneys. The Microtubule Plus-End Tracking Protein ARMADILLO-REPEAT KINESIN1 Promotes Microtubule Catastrophe in Arabidopsis . *The Plant Cell*, 26(8):3372–3386, 08 2014.

[15] Ezgi Can Eren, Ram Dixit, and Natarajan Gautam. A three-dimensional computer simulation model reveals the mechanisms for self-organization of plant cortical microtubules into oblique arrays. *Molecular Biology of the Cell*, 21(15):2674–2684, 2010. PMID: 20519434.

[16] Ezgi Can Eren, Natarajan Gautam, and Ram Dixit. Computer simulation and mathematical models of the noncentrosomal plant cortical microtubule cytoskeleton. *Cytoskeleton*, 69(3):144–154, 2012.

[17] Ilse Foissner and Geoffrey O. Wasteneys. Microtubules at wound sites of nitella internodal cells passively co-align with actin bundles when exposed to hydrodynamic forces generated by cytoplasmic streaming. *Planta*, 208(4):480–490, 6 1999.

[18] Olivier Hamant, Daisuke Inoue, David Bouchez, Jacques Dumais, and Eric Mjolsness. Are microtubules tension sensors? *Nature Communications*, 10(1), 2019.

[19] Olivier Hamant and Jan Traas. The mechanics behind plant development. *The New phytologist*, 185(2):369–385, January 2010.

[20] Regina Himmelspach, Richard E. Williamson, and Geoffrey O. Wasteneys. Cellulose microfibril alignment recovers from dcb-induced disruption despite microtubule disorganization. *The Plant Journal*, 36(4):565–575, 10 2003.

[21] Jonathon Howard. *Mechanics of motor proteins and the cytoskeleton*. Sinauer Associates, 2001.

[22] Bas Jacobs, Marco Saltini, Jaap Molenaar, Laura Filion, and Eva E. Deinum. Protoxylem microtubule patterning requires rop pattern co-alignment, realistic microtubule-based nucleation, and moderate microtubule flexibility. *bioRxiv*, 2024.

[23] Bas Jacobs, René Schneider, Jaap Molenaar, Laura Filion, and Eva E. Deinum. Microtubule nucleation complex behavior is critical for cortical array homogeneity *and* xylem wall patterning. *Proceedings of the National Academy of Sciences*, 119(50), 12 2022.

[24] Eiko Kawamura and Geoffrey O. Wasteneys. MOR1, the Arabidopsis thaliana homologue of Xenopus MAP215, promotes rapid growth and shrinkage, and suppresses the pausing of microtubules in vivo. *Journal of Cell Science*, 121(24):4114–4123, 12 2008.

[25] Marco Cosentino Lagomarsino, Catalin Tanase, Jan W. Vos, Anne Mie C. Emons, Bela M. Mulder, and Marileen Dogterom. Microtubule organization in three-dimensional confined geometries: Evaluating the role of elasticity through a combined in vitro and modeling approach. *Biophysical Journal*, 92(3):1046–1057, 2007.

[26] Lei Lei, Tian Zhang, Richard Strasser, Christopher M. Lee, Martine Gonneau, Lukas Mach, Samantha Vernhettes, Seong H. Kim, Daniel J. Cosgrove, Shundai Li, and Ying Gu. The*jiaoyao1*mutant is an allele of*korrigan1*that abolishes endoglucanase activity and affects the organization of both cellulose microfibrils and microtubules in*Arabidopsis* . *The Plant Cell*, 26(6):2601–2616, 6 2014.

[27] Jing Li, Daniel B. Szymanski, and Taeyoon Kim. Probing stress-regulated ordering of the plant cortical microtubule array via a computational approach. *BMC Plant Biology*, 23(1):308, Jun 2023.

[28] Jelmer J. Lindeboom, Antonios Lioutas, Eva E. Deinum, Simon H. Tindemans, David W. Ehrhardt, Anne Mie Emons, Jan W. Vos, and Bela M. Mulder. Cortical microtubule arrays are initiated from a nonrandom prepattern driven by atypical microtubule initiation. *Plant Physiology*, 161(3):1189–1201, Jan 2013.

[29] Jelmer J. Lindeboom, Masayoshi Nakamura, Marco Saltini, Anneke Hibbel, Ankit Walia, Tijs Ketelaar, Anne Mie Emons, John C. Sedbrook, Viktor Kirik, Bela M. Mulder, and et al. Clasp stabilization of plus ends created by severing promotes microtubule creation and reorientation. *Journal of Cell Biology*, 218(1):190–205, Jan 2018.

[30] Vincent Mirabet, Pawel Krupinski, Olivier Hamant, Elliot M. Meyerowitz, Henrik Jönsson, and Arezki Boudaoud. The self-organization of plant microtubules inside the cell volume yields their cortical localization, stable alignment, and sensitivity to external cues. *PLOS Computational Biology*, 14(2):1–23, 02 2018.

[31] Takashi Murata, Seiji Sonobe, Tobias I. Baskin, Susumu Hyodo, Seiichiro Hasezawa, Toshiyuki Nagata, Tetsuya Horio, and Mitsuyasu Hasebe. Microtubule-dependent microtubule nucleation based on recruitment of gamma-tubulin in higher plants. *Nature Cell Biology*, 7(10):961–968, Sep 2005.

[32] Masayoshi Nakamura, Jelmer J. Lindeboom, Marco Saltini, Bela M. Mulder, and David W. Ehrhardt. Spr2 protects minus ends to promote severing and reorientation of plant cortical microtubule arrays. *Journal of Cell Biology*, 217(3):915–927, Jan 2018.

[33] WASTENEYS G. O. Microtubule orientation in developing internodal cells of nitella : a quantitative analysis. *Eur. J. Cell Biol.*, 43:14–22, 1987.

[34] Frank Sainsbury, David A. Collings, Ken Mackun, John Gardiner, John D.I. Harper, and Jan Marc. Developmental reorientation of transverse cortical microtubules to longitudinal directions: a role for actomyosin-based streaming and partial microtubule-membrane detachment. *The Plant Journal*, 56(1):116–131, 2008.

[35] Marco Saltini and Eva E. Deinum. Microtubule simulations in plant biology: A field coming to maturity. *Current Opinion in Plant Biology*, 81:102596, 2024.

[36] Marco Saltini and Eva E. Deinum. A realistic algorithm for microtubule-based nucleation reveals large sensitivity to cell geometry of the plant cortical array. *bioRxiv*, 2024.

[37] Takema Sasaki, Kei Saito, Daisuke Inoue, Henrik Serk, Yuki Sugiyama, Edouard Pesquet, Yuta Shimamoto, and Yoshihisa Oda. Confined-microtubule assembly shapes three-dimensional cell wall structures in xylem vessels. *Nature Communications*, 14(1), 11 2023.

[38] René Schneider, Kris van't Klooster, Kelsey L. Picard, Jasper van der Gucht, Taku Demura, Marcel Janson, Arun Sampathkumar, Eva E. Deinum, Tijs Ketelaar, and Staffan Persson. Long-term single-cell imaging and simulations of microtubules reveal principles behind wall patterning during proto-xylem development. *Nature Communications*, 12(1):669, Jan 2021.

[39] Sidney L. Shaw, Roheena Kamyar, and David W. Ehrhardt. Sustained microtubule treadmilling in arabidopsis cortical arrays. *Science*, 300(5626):1715–1718, 2003.

[40] Xia-qing Shi and Yu-qiang Ma. Understanding phase behavior of plant cell cortex microtubule organization. *Proceedings of the National Academy of Sciences*, 107(26):11709–11714, Jun 2010.

[41] Cątąlin C. Tąnase. *Physical Modeling of Microtubule Force Generation and Self-organization*. Wageningen University, 2004.

[42] Tim Y. Y. Tian, Colin B. Macdonald, and Eric N. Cytrynbaum. A stochastic model of cortical microtubule anchoring and mechanics provides regulatory control of microtubule shape. *Bulletin of Mathematical Biology*, 85(11):103, Sep 2023.

[43] Simon Tindemans, Eva Deinum, Jelmer Lindeboom, and Bela Mulder. Efficient event-driven simulations shed new light on microtubule organization in the plant cortical array. *Frontiers in Physics*, 2, 2014.

[44] Simon H. Tindemans, Rhoda J. Hawkins, and Bela M. Mulder. Survival of the aligned: Ordering of the plant cortical microtubule array. *Physical Review Letters*, 104(5), 2010.

[45] Magalie Uyttewaal, Agata Burian, Karen Alim, Benoît Landrein, Dorota Borowska-Wykręt, Annick Dedieu, Alexis Peaucelle, Michał Ludynia, Jan Traas, Arezki Boudaoud, Dorota Kwiatkowska, and Olivier Hamant. Mechanical stress acts via katanin to amplify differences in growth rate between adjacent cells in arabidopsis. *Cell*, 149(2):439–451, 4 2012.

[46] Jan W. Vos, Marileen Dogterom, and Anne Mie Emons. Microtubules become more dynamic but not shorter during preprophase band formation: A possible "search-and-capture" mechanism for microtubule translocation. *Cell Motility and the Cytoskeleton*, 57(4):246–258, Jan 2004.

[47] Geoffrey O. Wasteneys and Moira E. Galway. Remodeling the cytoskeleton for growth and form: An overview with some new views. *Annual Review of Plant Biology*, 54(Volume 54, 2003):691–722, 2003.

[48] Geoffrey O. Wasteneys and Richard E. Williamson. Reassembly of microtubules in Nitella tasmanica: assembly of cortical microtubules in branching clusters and its relevance to steady-state microtubule assembly. *Journal of Cell Science*, 93(4):705–714, 08 1989.

[49] GO Wasteneys and RE Williamson. Reassembly of microtubules in nitella tasmanica: quantitative analysis of assembly and orientation. *European journal of cell biology*, 50(1):76–83, 1989.

[50] Raymond Wightman and Simon R. Turner. Severing at sites of microtubule crossover contributes to microtubule alignment in cortical arrays. *The Plant Journal*, 52(4):742–751, Sep 2007.