Supplemental Materials
Molecular Biology of the Cell

McCoy et al.
**Supplementary Material**

Table S1. Plasmids used in this study

| Plasmid name | Relevant marker/gene | Source          |
|--------------|----------------------|-----------------|
| pMG2343      | mCherry-NAT1         | This work       |
| pGEM-ARG4    | ARG4                 | Wilson et al., 1999 |
| pMG2120      | GFP-NAT1             | This work       |
| pMG2254      | mCherry-URA3         | This work       |
| pGEM-URA3    | URA3                 | Wilson et al., 1999 |
| pMG1646      | GFP-HIS1             | This work       |
| pGEM-HIS1    | HIS1                 | Wilson et al., 1999 |
| pMG1602      | GFP-URA3             | This work       |

Table S2. Primers used in this study

| Primer number | Primer purpose/location                      | Primer sequence                                                                                                                                 |
|---------------|----------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| 5033          | Forward primer to amplify mCherry-NAT1 to tag MTW1 | CATTGATATGATAATACCAGAACCAAGAGACGATATAGATGTGGATGCAATAAAAGAATATAATGCTCAAATTgtttaaaggtgaagaagataaatgg |
| 5034          | Reverse primer to amplify mCherry-NAT1 to tag MTW1 | CGATTAGTATTGATCTATTGTTTGTAACCTAAATCAACTTGTATTTTGGATCCAGAACCTTACCACTGgtgtggacctgcgtgcttgcacttac |
| 2717          | Forward primer within MTW1 ORF                | GCTAGTGGTGCTGGTGGAATGAAG                                                                                                                         |
| 4036          | Reverse primer within mCherry ORF             | CACCAGTTGAATGTCTACCTTCAGCTC                                                                                                                       |
|   | Forward primer | Reverse primer | Sequence                          | Description                                      |
|---|----------------|----------------|------------------------------------|-------------------------------------------------|
| 4368 | Forward primer to amplify ARG4 to delete KIP1 | Reverse primer to amplify ARG4 to delete KIP1 | CTCATAGCAGATTATCATCAATGTCAATAT CCAAGTTGGTTAGGTGCTCGAGGAAGGAA CTCCTCAAGgttttccagtcacgcgtt | Forward primer to amplify ARG4 to delete KIP1 | Reverse primer to amplify ARG4 to delete KIP1 | CTAGTATAAACCTCAAAATTTAAACATG TACGTGAAAAATGGAGTTAAACAAATATTG TCTAATTtggtgaattgtgacgggata |
| 4371 | Reverse primer to check deletion of KIP1 with ARG4 | Forward primer within ARG4 | GAACTATAAGGAGGAAAGGGAAGG | Reverse primer to check deletion of KIP1 with ARG4 | Forward primer within ARG4 | GTCGCAATGAAGAACCAGTGAATAAGC |
| 4732 | Forward primer to tag KIP1 with GFP-NAT | Reverse primer to tag KIP1 with GFP-NAT | ATAAAACAGATATAAACCTCAAAATATTAAACATG TACGTGAAAAATGGAGTTAAACAAATATTG TCTAATTtggtgaattgtgacgggata | Forward primer to tag KIP1 with GFP-NAT | Reverse primer to tag KIP1 with GFP-NAT | ATAAAACAGATATAAACCTCAAAATATTAAACATG TACGTGAAAAATGGAGTTAAACAAATATTG TCTAATTtggtgaattgtgacgggata |
| 730 | Reverse primer to check deletion of KIP1 with ARG4 | Forward primer within ARG4 | GTGGGTGTTGTTGCTACG | Reverse primer to check deletion of KIP1 with ARG4 | Forward primer within ARG4 | GTGGGTGTTGTTGCTACG |
| 658 | Reverse primer within GFP to check tagging | Forward primer within GFP to check tagging | TTTGTCACAATTCATCCATACCATG | Reverse primer within GFP to check tagging | Forward primer within GFP to check tagging | TTTGTCACAATTCATCCATACCATG |
| 5838 | Forward primer to tag TUB1 with mCherry-URA3 | Reverse primer to tag TUB1 with mCherry-URA3 | ATAAAACAGATATAAACCTCAAAATATTAAACATG TACGTGAAAAATGGAGTTAAACAAATATTG TCTAATTtggtgaattgtgacgggata | Forward primer to tag TUB1 with mCherry-URA3 | Reverse primer to tag TUB1 with mCherry-URA3 | ATAAAACAGATATAAACCTCAAAATATTAAACATG TACGTGAAAAATGGAGTTAAACAAATATTG TCTAATTtggtgaattgtgacgggata |
| 5839 | Reverse primer to tag TUB1 with mCherry-URA3 | Forward primer within URA3 | AGACCTATAGTGAGAGAGCA | Reverse primer to tag TUB1 with mCherry-URA3 | Forward primer within URA3 | AGACCTATAGTGAGAGAGCA |
| 944 | Reverse primer to check tagging of TUB1 | Forward primer to delete KIP3 with ARG4 | CCTCCTCTTTAACCATTGGACACACC | Reverse primer to check tagging of TUB1 | Forward primer to delete KIP3 with ARG4 | CCTCCTCTTTAACCATTGGACACACC |
| 6042 | Forward primer to delete KIP3 with ARG4 | Reverse primer to delete KIP3 with ARG4 | TAAATGAAAAACCCGACCTTTGTGATTAAAA AAAATTTAACATTAGCAACAAAGTGAAGAACA CGATCAAAttagaagaccacctttgattg | Forward primer to delete KIP3 with ARG4 | Reverse primer to delete KIP3 with ARG4 | TAAATGAAAAACCCGACCTTTGTGATTAAAA AAAATTTAACATTAGCAACAAAGTGAAGAACA CGATCAAAttagaagaccacctttgattg |
| 6043 | Reverse primer to check deletion of KIP3 with ARG4 | Forward primer within URA3 | GAAGAGATGATAGAAGAGAT | Reverse primer to check deletion of KIP3 with ARG4 | Forward primer within URA3 | GAAGAGATGATAGAAGAGAT |
| Primer ID | Description                                                                 | Primer Sequence |
|----------|-----------------------------------------------------------------------------|-----------------|
| 6056     | Forward primer to delete last 42 bases from KIP1 with URA3                  | ACAAAGATCAAAAAAGTAATGGCTCTGAAGATACATCACCACAAGATTCTACACGACCAATAATAATTGATGA ttccagtcacgagctgtgtaaaacga |
| 6057     | Reverse primer to delete last 42 bases from KIP1 with URA3                  | ATAAACAAGATATAAAACCTCACAATTAATTAAACATGTACTGAACAAATGGAGTAAAACAAATATTGGTC tgtgtaaggatgtgacgcggataaattaatccac |
| 945      | Reverse primer within URA3                                                  | CAAAACATCCTCTACCAACA |
| 5621     | Forward primer within KIP1                                                  | GTGAAATCTATTTGGGATACAAAC |
| 5624     | Reverse primer downstream of KIP1                                            | CCGCTCGAGGGAAATATGGAACTATAAGGA GG |
| 5345     | Reverse primer to tag TUB4 with mCherry-NAT1                                | GCGCAATATTAATCCACAACGGAAAAATGTT TTGACTCCACAACCAACAAAAAGTATTCCCTCA actccgacactggtgacgcgattgacttc |
| 4311     | Forward primer to tag TUB4 with mCherry-NAT1                                | GGATGACCTAGAAGATGGTGTTGGAATGG TAAATTGGTTATAAAATATAGATGATGCAGATATGGGTATAGGTGTGTgttccaaaaggtgaaagagatatt |
| 3326     | Reverse primer to check mCherry tag                                          | CGCGGATCCTTATTTATATAATTCAATCCACATAC CACCAGTTG |
| 4313     | Forward primer to check TUB4-mCherry tag                                     | GTGCCCTGGGGTAGCTCGATCCGTATTAG |
| 2715     | Forward primer to tag MTW1 with GFP-HIS1                                     | CATTGATATGATAATACCAGAAACAAGACGAT ATAGATGTGGATGCAATAAAAGAATATAAAATG CTCAAATTctaaagtgaaggaatttatt |
| 4266     | Reverse primer to tag MTW1 with GFP-HIS1                                     | GATACTCAATCTGAAGAACCACAATTTGTTT ACTTTATGGGAAGATTACATGAAAGATGGCAT CAAGCAAGaatccggaatatttagaatgaaac |
| 4312     | Reverse primer to tag TUB4 with mCherry-URA3                                | GCGCAATATTAATCCACAACGGAAAAATGTT TTGACTCCACAACCAACAAAAAGTATTCCCTCA ACTCGGACtctgaaggaccacctttgatttg |
| 565      | Reverse primer within HIS1                                                  | CTGCAGTACCAATATATCGGTTGC |
| 4370     | Forward primer to check deletion of KIP1                                     | CTTCCTTTATGTGGCCTGCAATAGTATTGTC |
| 6158     | Forward primer to tag KIP3 with GFP-URA3                                     | GATGAATTCTGATCTGACATACCAATTAAGATCTAACTTAAATAACAGCTGACATCACAATAAGTAAATAAT TAGATATctaaaggtgaaggaatttatt |
**Spindle length distributions for various cell lines**

The metaphase spindle length was determined by taking one standard deviation on either side of the mean spindle length (Figure S1A). The length of several spindles was tracked over time, and the spindles maintained a length around the average spindle length for a long period of time prior to a sudden lengthening event corresponding to anaphase (Figure S1B). The average spindle lengths with respect to Tub1-GFP of the various cell lines used is shown in figure S2. This shows a clear shortening of the metaphase spindle length in spindles with reduced numbers of Kip1p in the nucleus ($KIP1/kip1\Delta$ and $KIP1/kip1(NLS\Delta)$) relative to WT and an increase in spindle length of spindles with reduced numbers of Kip3p ($KIP3/kip3\Delta$), both of which are outcomes consistent with the model.

Figure S1A
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Figure S1B: Steady state spindle length defines metaphase spindles. A) Spindle length distributions of Tub1-GFP cell lines B) Spindle length time course for Tub1-GFP in WT spindles.
Figure S2: Mean spindle lengths measured from Tub1-GFP for each Tub1-GFP cell line

**Tub4 spindle lengths are consistent with Tub1-GFP spindle lengths**

Average spindle length measured relative to WT Tub4-mCherry and WT Tub4-GFP (γ-tubulin) is consistent with the average spindle length as measured relative to WT Tub1-GFP. γ-tubulin is an inner SPB protein and tagging it with either mCherry or GFP allows it to serve as a pole marker (Knop and Schiebel, 1997; Nguyen et al., 1998). The average spindle length in the WT Tub4-mCherry cells was measured to be 990 ± 10 nm (n = 99), and the WT Tub4-GFP spindles were measured to be 910 ± 10 nm (n = 54) as compared to 842 ± 6 nm in the WT Tub1-GFP cells (Figure S3). The longer spindle lengths measured using Tub4-GFP relative to Tub1-GFP are likely because γ-tubulin is located in the SPB, which is proximal to the spindle MTs. The longer spindle length measured using Tub4-mCherry relative to Tub4-GFP (p = 1.1 x 10⁻⁵) is likely due to the larger point spread function associated with the red fluorophore (Born and Wolf 1997). This means that γ-tubulin can be used as a marker to find in-plane spindles in
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lieu of α – tubulin in experiments where it is advantageous to use GFP as a marker for non α – tubulin proteins, such as described above for Cse4 and Kip3p.

Figure S3: The mean spindle lengths for the WT Tub4-mCherry spindles, WT Tub4-GFP spindles, and the WT Tub1-GFP spindles. Tub4-GFP/Tub4; Tub4-mCherry/Tub4; and Tub1-GFP/Tub1, MTW1-mCherry/MTW1.

Statistical Analysis

The p values for the fluorescence intensity distributions were calculated using a method to compare simulated random sum of square error (SSE) values to the experimentally determined SSE values. The SSE values were simulated by randomly shuffling all \((m + n)\) fluorescence intensity distributions in the experimental data set with \(m\) fluorescence intensity distributions for the WT cells and \(n\) fluorescence intensity distributions for the mutant cells. The first \(m\) shuffled distributions were set aside as the simulated wild type distributions and averaged, and the remaining \(n\) distributions were called the simulated mutant distributions and averaged. The SSE of the simulated wild type mean distribution and the simulated mutant mean distribution was calculated and stored. This was done 10,000 times, and the simulated SSEs were ranked. The SSE of the experimental data set was calculated and the rank of the experimental SSE was found with respect to the ranked simulated SSEs \((A)\). The p value was calculated as follows:

\[
p = \frac{10000 - U}{10000}
\]

(S1)

The p values for the spindle length comparisons were calculated using independent t-tests.
**Model Simulation Parameters**

Table S3 lists the model simulation parameters and the values used to obtain the results shown in figure 9 in the main text. These values are based on the values published in Gardner et al. 2008.

**TABLE S3: Model simulation parameters and values**

| Parameter description          | Symbol | C. albicans WT parameter values | C. albicans KIP1/kip1 Δ parameter values | S. cerevisiae WT parameter values | S. cerevisiae cin8Δ Parameter values | Units  |
|-------------------------------|--------|---------------------------------|----------------------------------------|----------------------------------|-------------------------------------|--------|
| Growth velocity               | V_g    | 1.2                             | 1.2                                    | 1.2                              | 1.9                                 | µm/min |
| Shortening velocity           | V_s    | 1.2                             | 1.2                                    | 1.2                              | 1.9                                 | µm/min |
| Chromatin spring constant     | ρ      | 0.9                             | 0.9                                    | 0.9                              | 0.9                                 | µm⁻¹   |
| Basal rescue frequency        | k_r,0  | 9                               | 9                                      | 9                                | 9                                   | min⁻¹  |
| Basal rescue frequency (iMTs) | k_r,0_iM T | 60                          | 60                                     | 60                               | 60                                  | min⁻¹  |
| Basal catastrophe frequency   | k_c,0  | 2                               | 2                                      | 2                                | 2                                   | min⁻¹  |
| Catastrophe sensitivity factor| β_m    | 20                              | 20                                     | 20                               | 20                                  | min⁻¹  |
| Number of motors              | N_m    | 30                              | 10                                     | 90                               | 20                                  | --     |
Chromosome alignment during metaphase, also known as congression, is mediated by the dynamics of kinetochore microtubule plus ends (Goshima & Scholey, 2010b; Inoué & Salmon, 1995). As kinetochore microtubules assemble and disassemble, they move kinetochores, which are physical attachment sites between chromosomes and dynamic microtubule plus ends (Gardner et al., 2008; Maddox et al., 2000; Skibbens et al., 1993). A key feature of mitotic spindles is that they typically arrive at a congressed state, although with some degree of noise.

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| Parameter                                                                 | Symbol | Value 1 | Value 2 | Value 3 | Value 4 |
|--------------------------------------------------------------------------|--------|---------|---------|---------|---------|
| Unloaded motor velocity                                                  | \( v_u \) | 100     | 100     | 100     | 100     | nm/sec |
| Motor stall force                                                        | \( F_{\text{stall}} \) | 6       | 6       | 6       | 6       | pN     |
| Motor spring constant                                                    | \( \rho_m \) | 0.5     | 0.5     | 0.5     | 0.5     | pN/nm  |
| Unloaded motor off-rate constant                                         | \( k_{\text{off}} \) | 0.3     | 0.3     | 0.3     | 0.3     | sec\(^{-1}\) |
| Motor on-rate constant                                                   | \( k_{\text{on}} \) | 1       | 1       | 1       | 1       | \( \mu M^{-1}\text{sec}^{-1} \) |
| Radius of 2\(^{nd}\) motor head attach point relative to 1\(^{st}\) motor head | \( r_M \) | 40      | 40      | 40      | 40      | Nm     |
| Critical force                                                           | \( F_c \) | 6       | 6       | 6       | 6       | pN     |
| Spindle length                                                           | --     | 0.84    | 0.77    | 1.6     | 1.4     | \( \mu m \) |

Derivation of dimensionless number based on Péclet number for precision in chromosome congression in the presence of kinetochore microtubule assembly/disassembly noise.
in the positioning, due to so-called directional instability (Skibbens et al., 1993). The positioning can be characterized by a signal-to-noise ratio (SNR) given by

\[ SNR = \frac{\langle L \rangle}{\sigma} \]  

(S2)

where \( \langle L \rangle \) is the mean kinetochore microtubule length and \( \sigma \) is the standard deviation of the length as illustrated in Figure 1. Another common definition of the relative strength of noise is the coefficient of variation (CV), which is defined by

\[ CV = \frac{\sigma}{\langle L \rangle} = \frac{1}{SNR} \]  

(S3)

In the limit of perfect congression, \( \sigma = 0 \), and so \( CV = 0 \) and \( SNR \to \infty \). However, in the limit of no motors, and therefore no length control, microtubule lengths will be exponentially distributed, in which case \( \langle L \rangle = \sigma \) (Odde & Buettner, 1998; Odde et al., 1995). Therefore, a fundamental limit for achieving congression is the following inequality

\[ CV < 1 \]  

(S4)

As a consequence, it would be useful to determine the CV as a function of the microtubule assembly parameters, including the motor-mediated catastrophe, which leads to an increase in catastrophe with increasing length (Gardner et al., 2005, 2008; Sprague et al., 2003; Varga et al., 2006). Using the microtubule dynamics model described above, where the catastrophe frequency increases with kMT length according to a constant, \( \beta \), there will be an “attractor” point, where the catastrophe frequency exceeds the rescue frequency and this attractor will be approximately located at \( \langle L \rangle \). As depicted in Figure S4, if a kMT grows longer than \( \langle L \rangle \), it will tend to undergo net disassembly, and if it is shorter than \( \langle L \rangle \), it will tend to undergo net assembly. Physically, this is due to the increased binding of motors and their transport to the plus end, which obeys a linear relationship between motor density and the growing tip and microtubule length (Gardner et al., 2008; Varga et al., 2006).
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Figure S4: The microtubule dynamics model predicts an attractor zone where the catastrophe frequency and the rescue frequency converge.

To estimate $\langle L \rangle$, we first define the drift velocity of kMT plus ends as (Seetapun & Odde, 2010)

$$V_D = \frac{\langle L_g \rangle - \langle L_s \rangle}{t_g + t_s} \quad (S5)$$

where $\langle L_g \rangle = V_g/k_c$ is the mean growth length, $\langle L_s \rangle = V_s/k_r$ is the mean shortening length, $t_g = 1/k_c$ is the mean growth time, and $t_s = 1/k_r$ (note the $V_g$, $V_s$, $k_c$, and $k_r$ are the usual four parameters of dynamic instability that define the net growth rate, the net shortening rate, the catastrophe frequency, and the rescue frequency, respectively). In the case of length-dependent catastrophe, we can linearly approximate the catastrophe frequency as

$$k_c(x) = \beta x \quad (S6)$$

where $x$ is the distance from the kMT plus end to its minus end (located at the spindle pole), and $\beta$ is the strength of the length-dependent catastrophe. Substituting, Eqn. 6 into Eqn. S5, we obtain a position-dependent drift velocity

$$V_D(x) = \frac{V(1-t_s\beta x)}{1+t_s\beta x} \quad (S7)$$
where \( V = V_g = V_s \) (assumes that \( V_g \approx V_s \); see Gardner et al., 2008; Table S3). Let \( x_1 \) define the position of the attractor for kMT plus ends, which is also the value of \( x \) at which the drift velocity is zero

\[
V_D(x_1) = 0 \quad (S8)
\]

which, when combined with Eqn. S7, yields

\[
x_1 = \frac{1}{\tau_s \beta} . \quad (S9)
\]

Since \( x_1 \approx <L> \), we thus have the following relationship for the mean kMT length

\[
\langle L \rangle = \frac{1}{\beta \tau} \quad (S10)
\]

where \( \tau = t_g(x_1) = t_s \), is the mean time for catastrophe at the attractor position, or equivalently the mean time for rescue (since \( t_g(x_1) = t_s \) at the attractor position \( x_1 \)).

To estimate \( \sigma \), we first consider the kMT plus end as if it were a thermally driven spring, with rest position defined by the plus end attractor position, \( x_1 \) (i.e. the value of \( x \) where \( V_D(x) = 0 \), or equivalently when \( x = <L> \)), so that by the Equipartition Theorem (Howard, 2001)

\[
\sigma^2 = \frac{k_B T}{\kappa} \quad (S11)
\]

where \( k_B \) is Boltzmann’s constant, \( T \) is the absolute temperature, and \( \kappa \) is the spring constant.

The spring constant here describes an “effective” spring that acts to restore the kMT plus end to its mean length via the length-dependent catastrophe. To obtain an expression for \( \kappa \), we can start by writing a force balance for this system as

\[
\sum F = ma = -\kappa (x - x_1) - \gamma v . \quad (S11)
\]

Assuming an overdamped system (\( m = 0 \)), solving for \( v \), and substituting the drift velocity, we obtain

\[
V_D(x) = -\frac{\kappa (x - x_1)}{\gamma} . \quad (S12)
\]
Substituting for the drift velocity (Eqn. S7), and the catastrophe gradient strength for $x_1$ (Eqns. S9 and S10), we obtain

$$V(1-t_s\beta x) \left(1 + t_s\beta x\right) = -\kappa \left(x - \frac{1}{t_s\beta}\right) \gamma^{-1}. \quad \text{(S13)}$$

Multiplying both sides by $t_s\beta\gamma$, Eqn. S13 becomes

$$t_s\beta V(1-t_s\beta x) \left(1 + t_s\beta x\right) = -\kappa (t_s\beta x - 1) \quad \text{(S14)}$$

which allows us to solve for $\kappa$, given by

$$\kappa = \frac{t_s\beta V}{1 + t_s\beta x} \quad \text{(S15)}$$

near $x=x_1=<L>$, we can use Eqns. S9 and S10 to obtain

$$\kappa = \frac{\gamma V \beta \tau}{2} \quad \text{(S16)}$$

Substituting Eqn. S16 into Eqn. S11, the variance in tip position can now be written as

$$\sigma^2 = \frac{2k_BT}{\gamma V \beta \tau} \quad \text{(S17)}$$

which can be further simplified by defining a diffusion coefficient, $D=k_BT/\gamma$, to yield

$$\sigma^2 = \frac{2D}{V \beta \tau} \quad \text{(S18)}$$

The diffusion coefficient, $D$, represents the “noise” in microtubule dynamic instability, which has been previously derived by Maly (2002; Eqn. 15) in terms of the four parameters of dynamic instability as

$$D = \frac{k_c k_r (V_s + V_c)^2}{(k_c + k_r)^3}. \quad \text{(S19)}$$
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In the present case, the catastrophe frequency is modeled as a linear function of $x$ (Eqn. S6), and also we assume that $V=V_g=V_s$ (justified above for Eqn. S7), so that

$$D(x) = \frac{\beta x_k r (2V)^2}{(\beta x + k_r)^3}.$$  \hspace{1cm} (S20)

As described above, when $x=x_1=<L>$, then $\tau=\tau_0(x_1)=t_s$, meaning that $k_c(x_1)=k_r=\beta x_1$, so that we can rewrite Eqn. S20 as

$$D(x_1) = \frac{(\beta x_1)^2 (2V)^2}{(2 \beta x_1)^3}.$$  \hspace{1cm} (S21)

or equivalently,

$$D(x_1) = \frac{V^2}{2 \beta x_1}.$$  \hspace{1cm} (S22)

Since $\tau=1/\beta x_1$, we can rewrite Eqn. S22 as

$$D(x_1) = \frac{\tau V^2}{2}.$$  \hspace{1cm} (S23)

Returning now to the CV, or equivalently the SNR, as a metric for congression quality, Eqns. S10 and S18 can be combined to obtain

$$\frac{\sigma^2}{\langle L \rangle^2} = \frac{\left(\frac{2D}{V \beta \tau}\right)}{\left(\frac{1}{\beta \tau}\right)^2}.$$  \hspace{1cm} (S24)

which simplifies to become

$$\frac{\sigma^2}{\langle L \rangle^2} = \frac{2D \beta \tau}{V}.$$  \hspace{1cm} (S25)

Again, substituting in Eqn. S10, we obtain
The dimensionless quantity on the right-hand side of Eqn. S26 can be rewritten as

\[ \frac{\sigma^2}{\langle L \rangle^2} = \frac{2}{Pe} \]  

(S27)

where

\[ Pe = \frac{V\langle L \rangle}{D} \]  

(S28)

with \( Pe \) representing the dimensionless quantity in convection-diffusion problems known as the Péclet number, which quantifies the strength of convective transport (\( V \)) relative to diffusive transport (\( D \)) for a system of a given size (\( <L> \)) (see Griffin et al., 2011). Therefore, the CV is given by

\[ \frac{\sigma}{\langle L \rangle} = \sqrt{\frac{2}{Pe}} . \]  

(S29)

We can rewrite \( Pe \) in terms of the fundamental microtubule assembly parameters, using Eqns. S10 and S23, as

\[ Pe = \frac{V(\beta \tau)^{-1}}{\left( \frac{\tau V^2}{2} \right)} \]  

(S30)

which simplifies to

\[ Pe = \frac{2}{V \beta \tau^2} \]  

(S31)

and the CV becomes

\[ \frac{\sigma}{\langle L \rangle} = \sqrt{V \beta \tau} . \]  

(S32)
In practice, we note that for finite spindle length, the distribution of plus-ends within the spindle will be approximately uniform, which has \( CV = \frac{1}{\sqrt{3}} = 0.58 \) (assuming \( V_D \sim 0 \)). Therefore, in practice for finite spindles, we do not expect to observe \( CV > 0.58 \) even in the absence of kMT length control (unless \( V_D < 0 \)).

Supplementary Brief Discussion on the Limits of Prokaryotic Genome Segregation

Recent studies suggest that there are fundamental limits to high fidelity chromosome segregation across phylogeny at around \( \sim 1 \) \( \mu m \) length scales. In the case of Ptacin et al., (2010), they investigated plasmid segregation in \( C. \) crescentus, and articulated a “burnt-bridge Brownian ratchet” mechanism for segregation. Here it seems that the fundamental prokaryotic spindle length is limited by the cell length, which is fundamentally different from the eukaryotic spindles, which often are much smaller than the cell size, even in smaller cells like \( C. \) albicans. Therefore, we suggest that bacterial segregation is largely limited by cell size, and that eukaryotic segregation is largely limited by spindle size. In the study of Vecchiarelli et al. (2014), a “diffusion-ratchet model” was proposed. The fundamental limits to length scales of such reaction-diffusion systems have been discussed previously in our modeling work (Lipkow and Odde, 2008) where the kinetics of the deactivating enzyme (e.g. phosphatase) limit the length of gradient to \( \sim 0.1-1 \) \( \mu m \). Thus, prokaryotic cell sizes need to be roughly one order of magnitude larger than the gradient length scale to exploit this mechanism, i.e. 1-10 \( \mu m \) (or equivalently Thiele modulus \( \sim 10 \); see Eqn. 2 in Lipkow and Odde), consistent with typical prokaryotic cell sizes.

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