

Supplementary Materials  
*Molecular Biology of the Cell*  
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# Interrelated effects of chromosome size, mechanics, number, location-orientation and polar ejection force on the spindle accuracy: a 3D computational study

## Supplementary Information

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## Supplementary Methods

**Reaction-Diffusion Master Equation formalism:** In the Stochastic Reaction-Diffusion-Dynamics model (SRDDM), we employed the Reaction-Diffusion Master equation (RDME) approach to model the biochemical reactions and molecular transport in a eukaryotic cell (Gardiner *et al.*, 1976; Isaacson, 2009; Isaacson and Isaacson, 2009). The volume ( $\sim 850 \mu\text{m}^3$ ) of the ellipsoidal shaped cell is divided into a large number of small subvolume elements (called subcells) of dimension  $l_{SV} = 0.25 \mu\text{m}$  (see Table S2). All the biomolecules within the cell are distributed among a large number of small subcells (Figure 1 in the main text). Chemical reactions are allowed only between molecules within a subcell. The molecules diffuse randomly between the next-neighbor subcells. The state of the cell  $\mathbf{X}$  is specified by the number of molecules and molecular complexes (e.g. KT-MT attachments)  $x_{j,v}$  of each type  $j = 1, 2, \dots, J$  in each subcell  $v = 1, 2, \dots, V$ . The time evolution of the probability distribution for the cell to be in state  $\mathbf{X}$  is given by the sum of contributions from all chemical reactions (reaction operator  $\mathbf{R}$ ), and from all diffusion events (diffusion operator  $\mathbf{D}$ ), i.e.

$$\begin{aligned} \frac{dP(\mathbf{X}, t)}{dt} &= \mathbf{R}P(\mathbf{X}, t) + \mathbf{D}P(\mathbf{X}, t) \\ &= \sum_v \sum_{\mu}^M [\alpha_{\mu}(\mathbf{x}_v - \mathbf{S}_{\mu})P(\mathbf{x}_v - \mathbf{S}_{\mu}, t) - \alpha_{\mu}(\mathbf{x}_v)P(\mathbf{x}_v, t)] \\ &\quad + \sum_v \sum_{\xi} \sum_j^J [d_j(\mathbf{x}_{j,v+\xi} + 1)P((\mathbf{x} + 1)_{j,v+\xi} - 1_{j,v}, t) - d_j \mathbf{x}_{j,v} P(\mathbf{x}_{j,v}, t)] \end{aligned} \quad (\text{S1})$$

The second line in Eq. (S1) describes the rate of change of  $P(\mathbf{X}, t)$  due to chemical reactions with  $\mathbf{x}_v$  being the column vector containing information about the number of molecules in the  $v$ -th subcell, with  $\alpha_{\mu}(\mathbf{x}_v)$  being the reaction propensity for the  $\mu$ -th reaction ( $\mu = 1, 2, \dots, M$ ) to occur in the  $v$ -th subcell, and with  $\mathbf{S}_{\mu}$  denoting the  $\mu$ -th column of  $J \times M$  stoichiometry matrix  $\mathbf{S}$  to describe the changes in the number of molecules when the  $\mu$ -th reaction occurs. The third line in Eq. (S1) describes the rate of change of  $P(\mathbf{X}, t)$  due to molecular diffusion events with  $d_j$  being the diffusion propensity for a molecule of type  $j$  to move from subcell  $v$  to the next-neighbor subcell  $v + \xi$ , where  $\xi$  is a next-neighbor subcell in the  $\pm x$ ,  $\pm y$ , and  $\pm z$  direction (total of 6 next-neighbor subcells) denoted by the unit vectors  $\mathbf{i}, \mathbf{j}, \mathbf{k}$ , and with  $x_{j,v}$  being the number of molecules of type  $j$  in subcell  $v$  (here  $1_{j,v}$  represents a single molecule of type  $j$  in subcell  $v$ ).

In SRDDM, the RDME (Eq. (S1) above) is sampled numerically using the Gillespie approach, which is based on the propensities of chemical reactions used (Gillespie, 1976, 1977). In the Gillespie approach, the probability that the  $\mu$ -th reaction will occur within the next time interval between  $t + \tau$

and  $t + \tau + dt$  is given by  $P_0(t + \tau)c_\mu h_\mu dt$ , where  $P_0(t + \tau)$  is the probability that at time  $t + \tau$  no reaction has occurred in the previous time interval  $(t, t + \tau)$ . The reaction propensity for the  $\mu$ -th reaction is given by the propensity  $\alpha_\mu = c_\mu h_\mu$ , and total propensity for all  $M$  reactions is  $\alpha_0 = \sum_{\mu=1}^M \alpha_\mu$  (Gillespie, 1976, 1977). The diffusion propensity for a molecule of type  $j$  ( $j = 1, 2, \dots, J$ ) to move from subcell  $v$  to the next-neighbor subcell  $v + \xi$  is given by  $d_j = \frac{D_j}{l_{sv}^2}$ , where  $D_j$  is the diffusion constant (see Tables S1, S2). In the SRDDM, the slowest diffusing particles are Aurora A and B, for which the diffusion timescale is  $\tau_D = 4.3 \times 10^{-4}$  s, and the most rapid reaction is MT-Ndc80 complex formation, for which the characteristic time is  $\tau_R = 2.6 \times 10^{-3}$  s. Therefore, the diffusion timescale is  $\sim 10$ -fold shorter than the characteristic reaction time,  $\tau_R \gg \tau_D$ . In this diffusion-controlled limit, the RDME formalism asymptotically approximates the Smoluchowski diffusion-limited reaction method (Elf and Ehrenberg, 2004; Erban and Chapman, 2009; Isaacson, 2009; Isaacson and Isaacson, 2009). In the SRDDM, we adapted the multi-particle diffusion (MPD) method (Roberts *et al.*, 2013) and the next-subvolume method (NSM) (Elf and Ehrenberg, 2004; Hattne *et al.*, 2005) for sampling Eq. (S1).

**Bead-spring and cylinder representation:** In the SRDDM, to describe the mechanical and force-generating processes within the mitotic spindle assembly, we introduce mechanical energies and forces for the mechanically active components (i.e. MTs, KTs, CHs), including interaction between them, for the non-covalent bonds they form (i.e. KT-MT, MT-CK, CH-CK linkages), and the cell boundary (see Table 1 in the main text). All the force-field parameters for all these components, except for CKs, are described in our previous study (Kliuchnikov *et al.*, 2022). The force field parameters related to CKs are described (Tables S1, S2). The particles described by a single interaction center (bead) are centrosomes and KTs. The *sister KT pair* is described by a pair of beads (two interaction centers) connected by a harmonic spring with the spring constant  $3.3 \times 10^3$  pN/nm (Table S2). Other components are represented by cylinders or by several beads connected by harmonic springs. Cylinders can be slightly stretched along their length; also the cylinder chain forming a filament (such as MT) can undergo bending. And finally, the effect of excluded volume interaction between two cylinders is also taken into account. All these interactions can be collected and described by cylinder potential (see Figure 1C in the main text):

$$\begin{aligned}
 U_{cyl} &= U_{cyl}^{str} + U_{cyl}^{bend} + U_{cyl}^{rep} \\
 &= \sum_i \frac{1}{2} K_r (r_i - r_0)^2 + \sum_{i,i+1} \frac{1}{2} K_\theta (\theta_{i,i+1} - \theta_0)^2 \\
 &\quad + \sum_{ij} \varepsilon \left( \frac{\sigma_c}{|\mathbf{r}_i + u(\mathbf{r}_{i+1} - \mathbf{r}_i) - \mathbf{r}_j + s(\mathbf{r}_{j+1} - \mathbf{r}_j)|} \right)^{12} \tag{S2}
 \end{aligned}$$

In Eq. (S2) stretching stiffness and bending rigidity  $K_r$  and  $K_\theta$ , for all the cylindrical components are given in Table S2, as well as equilibrium distance  $r_0$  and equilibrium angle  $\theta_0$ .  $\varepsilon$  is the energy scale, and  $\sigma_c$  is the length scale for the cylindrical excluded volume interaction,  $U_{cyl}^{rep} = 0$  when  $\sigma_c = R_{i,c} + R_{j,c}$ .  $u \in [0,1]$  and  $s \in [0,1]$  are the position factors. Repulsive potential for cylinders is described in detail below. An MT is described by a number of cylinders, connected in series. Each MT filament is described by the stretching stiffness 16.7 pN/nm and bending rigidity  $7.7 \times 10^5$  kJ/mol·rad<sup>2</sup>. We describe the CH arms by using 4-8 cylinders for each arm, depending on the 4-6  $\mu$ m length of the arm. The CH arms are described by the stretching stiffness  $3.3 \times 10^3$  pN/nm, and bending rigidity  $2.5 \times 10^5$  kJ/mol·rad<sup>2</sup>. The KT surface is modeled by a grid of particles of radius 4 nm. There are 750 beads per corona surface connected to each other by harmonic springs with the spring constant  $3.1 \times 10^2$  pN/nm

contained within the sphere of radius 200 nm. Selected beads in the KT surface are also connected to the KT center (virtual particle of radius 4 nm) by a harmonic spring with the spring constant  $3.3 \times 10^3$  pN/nm. In *CellDynaMo*, we also use the bead-spring representation to model the Ndc80 mediated KT-MT attachments. When an MT bumps into KT, Ndc80 can form a link between the plus-end of a growing MT (last bead of MT) and the closest bead on the KT surface within a sphere of radius 65 nm, which is the length of Ndc80. Ndc80 is modeled as a harmonic spring with the spring constant  $3.1 \times 10^2$  pN/nm. A bead-spring representation was also used to model Chromokinesin (CK) that appears on the surface of a chromosome arm when an MT is at a sufficiently close distance to the CH. One of the beads appears on the surface of the CH arm, and another bead appears on the surface of the MT. The spring, representing the linkage between the beads, has the stretching stiffness 16.7 pN/nm and the resting length of 50 nm.

**Force field for mechanical and force-generating components:** In the SRDDM, a cell configuration  $\mathbf{r}$  is specified in terms of the positions of mechanically active components and in terms of the interactions of these components,  $\mathbf{r} = \mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_i, \dots, \mathbf{r}_j, \dots, \mathbf{r}_N$ , where  $N$  is the total number of components. The total potential energy function for a cell configuration  $U(\mathbf{r})$  is given by the sum of potential energies for all mechanically active components and all the attachments:

$$\begin{aligned}
U(\mathbf{r}) = & \sum_i^N U_{MT}(\mathbf{r}_{MT,i}) + \sum_{i,j}^N U_{CH}(\mathbf{r}_{CH,i}, \mathbf{r}_{CH,j}) + \sum_{i,j}^N U_{KT}(\mathbf{r}_{KT,i}, \mathbf{r}_{KT,j}) \\
& + \sum_{i,j,k}^N U_{CK}(\mathbf{r}_{CK,i}, \mathbf{r}_{MT,j}, \mathbf{r}_{CH,k}) + \sum_{i,j}^N U_{att}(\mathbf{r}_{MT,i}, \mathbf{r}_{KT,j}) + \sum_{i,j}^N U_{rep}(\mathbf{r}_i, \mathbf{r}_j) \\
& + \sum_i^N U_{mem}(\mathbf{r}_i)
\end{aligned} \tag{S3}$$

Some potential energy terms ( $U_{MT}$ ,  $U_{CH}$ ,  $U_{KT}$  and  $U_{att}$ ) in Eq. (S3) were described in the previous work for bead-spring representation (Kliuchnikov *et al.*, 2022). With the introduction of the new component (CK) and the addition of cylinder representation, the total potential energy function includes two new energy terms: potential energy of interactions between CK and related MT and CH  $U_{CK}$  and excluded volume interactions between various components  $U_{rep}$ .  $U_{MT}$ ,  $U_{CH}$ ,  $U_{KT}$ ,  $U_{CK}$  and  $U_{att}$  are the potential energies of all MTs, CHs, KTs, CKs stretching and bending, and all CK-MT, CK-CH and KT-MT attachments, respectively;  $U_{rep}$  and  $U_{mem}$ , represent excluded volume interactions between various components (including spheres and cylinders) and the cell boundary (see below).

*MT filaments:* Each MT is described by the stretching potential with the distance  $r_{MT,ij}$  between the beginning and the end of the  $i$ -th cylinder and bending potential with the bending angle formed between the  $i$ -th and  $i + 1$ -th cylinders,  $\theta_{MT,i,i+1}$ ,

$$U_{MT} = U_{MT}^{str} + U_{MT}^{bend} = \sum_i \frac{1}{2} K_{MT,r} (r_{MT,i} - r_{MT,0})^2 + \sum_i \frac{1}{2} K_{MT,\theta} (\theta_{MT,i,i+1} - \theta_{MT,0})^2 \tag{S4}$$

In Eq. (S4)  $K_{MT,r}$  and  $K_{MT,\theta}$  are the stretching stiffness and bending rigidity for an MT (Table S2),  $r_{MT,0}$  is the equilibrium distance (cylinder' length) which depends on the length of an MT filament, and  $\theta_{MT,0} = 180^\circ$  is the equilibrium bending angle between adjacent cylinders (Table S2). For each cylinder in a MT structure, except for the last,  $r_{MT,0} = L_{cyl,max} = 1 \mu\text{m}$  (see Table S2), and  $r_{MT,0}$  for

the last cylinder is defined as the remainder of dividing the total microtubule length  $l_{MT}$  by  $L_{cyl,max}$  (see Table S2).

*Sister KT pair:* A sister KT pair is described by the stretching potential with the bead-to-bead distance  $r_{KT,ij}$ , stretching potential in the KT grid with the bead-to-bead distance  $r_{Ndc,ij}$ , and stretching potential between KT and beads in the KT grid with the bead-to-bead distance  $r_{KT-Ndc,ij}$ ,

$$\begin{aligned}
U_{KT} &= U_{KT}^{str} + U_{Ndc}^{str} + U_{KT,Ndc}^{str} \\
&= \sum_{ij} \frac{1}{2} K_{KT,r} (r_{KT,ij} - d_{KT})^2 + \sum_{ij} \frac{1}{2} K_{KT,r} (r_{Ndc,ij} - r_{Ndc,0})^2 \\
&+ \sum_{ij} \frac{1}{2} K_{KT,r} (r_{KT-Ndc,ij} - r_{KT-Ndc,0})^2
\end{aligned} \tag{S5}$$

In Eq. (S5)  $K_{KT,r}$  is the stretching stiffness for the sister KT pair and beads on the KT surface (Table S2), and  $d_{KT} = 725$  nm,  $r_{Ndc,0} = 8-200$  nm and  $r_{KT-Ndc,0} = 362.5-400$  nm are, respectively, the equilibrium distance between the sister KT beads (Table S2), equilibrium distance between beads in the KT grid (beads have different values of  $r_{Ndc,0}$  because bonds between them are formed within the sphere of radius  $R_{Ndc-bond} = 200$  nm), and equilibrium distance between the beads in the KT grid and the center of KT (all beads have different  $r_{KT-Ndc,0}$  values).

*CH arms:* Each flexible CH arm is described by the stretching potential with the distance  $r_{CH,i}$  and bending potentials with the bending angle  $\theta_{CH,ijk}$  within a single CH, and stretching potential with the distance  $r_{coh,ij}$  between the corresponding cylinders' centers of mass of the two sister CHs, (see Figure 1 in the main text),

$$\begin{aligned}
U_{CH} &= U_{CH}^{str} + U_{CH}^{bend} + U_{coh}^{str} \\
&= \sum_i \frac{1}{2} K_{CH,r} (r_{CH,i} - r_{CH,0})^2 + \sum_{i,i+1} \frac{1}{2} K_{CH,\theta} (\theta_{CH,i,i+1} - \theta_{CH,0})^2 \\
&+ \sum_{ij} \frac{1}{2} K_{coh,r} (r_{coh,ij} - r_{coh,0})^2
\end{aligned} \tag{S6}$$

In Eq. (S6)  $K_{CH,r}$ ,  $K_{CH,\theta}$  and  $K_{coh,r}$  are, respectively, the stretching stiffness and bending rigidity for CH arms, and stretching stiffness for sister CHs due to cohesin rings (see Table S2);  $r_{CH,0} = 725$  nm,  $\theta_{CH,0} = 180^\circ$  and  $r_{coh,0} = 725$  nm are, respectively, the equilibrium distance (cylinder' length) and equilibrium bending angle for beads within a single CH, and equilibrium distance between corresponding cylinders' centers of mass in the two sister CHs (Table S2).

*KT-MT attachments:* We describe the KT-MT interactions (for the MT plus-end linked to KT by Ndc80) using the harmonic potential with the distance  $r_{MT-Ndc,ij}$  between a bead at the MT plus-end and a bead in the KT:

$$U_{att} = \sum_{ij} \frac{1}{2} K_{Ndc,r} (r_{MT-Ndc,ij} - l_{Ndc})^2 \tag{S7}$$

In Eq. (S7)  $K_{Ndc,r}$  is the stretching stiffness and  $l_{Ndc} = 65.0$  nm is the equilibrium length of the Ndc80 linker (Table S2).

*Chromokinesin*: Each chromokinesin motor is described by stretching potentials with the bead-to-bead distance between the  $i$ -th CH bead and  $j$ -th MT bead  $r_{CK,i}$ , stretching potentials between the  $i$ -th MT bead and  $j$ -th MT cylinder end and  $j + 1$ -th MT cylinder end, stretching potentials between the  $i$ -th CH bead and  $k$ -th CH cylinder end and  $k + 1$ -th CH cylinder end with the distances  $r_{CK-MT,ij}$ ,  $r_{CK-MT,ij+1}$ ,  $r_{CK-MT,ik}$  and  $r_{CK-MT,ik+1}$ , respectively, and angles between the binding points and MT or CH cylinder axes  $\theta_{ij}$ ,  $\theta_{ij+1}$ ,  $\varphi_{ik}$  and  $\varphi_{ik+1}$ ,

$$\begin{aligned}
U_{CK} &= U_{CK}^{str} + U_{CK-MT}^{str} + U_{CK-CH}^{str} \\
&= \sum_i \frac{1}{2} K_{CK,r} (r_{CK,i} - r_{CK,0})^2 + \sum_{ij} \frac{1}{2} (\alpha K_{CK,r} (r_{CK-MT,ij} \cdot \cos[\theta_{ij}] - (1 - \alpha) l_{MT,j})^2 \\
&\quad + (1 - \alpha) K_{CK,r} (r_{CK-MT,ij+1} \cdot \cos\theta_{ij+1} - \alpha l_{MT,j})^2) \\
&\quad + \sum_{ik} \frac{1}{2} (\beta K_{CK,r} (r_{CK-CH,ik} \cdot \cos[\varphi_{ik}] - (1 - \beta) l_{CH,k})^2 \\
&\quad + (1 - \beta) K_{CK,r} (r_{CK-CH,ik+1} \cdot \cos\varphi_{ik+1} - \beta l_{CH,k})^2) \tag{S8}
\end{aligned}$$

In Eq. (S8)  $K_{CK,r} = 16.7$  pN/nm is the stretching stiffness for CK and  $r_{CK,0} = 50$  nm is the equilibrium distance of CK (Table S2).  $\alpha \in [0,1]$  and  $\beta \in [0,1]$  are the position factors that determine the fractional position of the motor on an MT cylinder and an CH cylinder, respectively (Popov *et al.*, 2016).

*Excluded volume interactions*: Excluded volume interactions might occur between any pairs of mechanically active components. There are two types of particles and so there are two types of excluded volume interactions: spherical (Lennard-Jones potential with the inter-particle separation distance  $r_{ij}$ ) and cylindrical. In the case of cylinders, forces are acting on the end points of both cylinders  $\mathbf{r}_i$ ,  $\mathbf{r}_{i+1}$ , and  $\mathbf{r}_j$ ,  $\mathbf{r}_{j+1}$  proportionally factors  $u \in [0,1]$  and  $s \in [0,1]$  that determine the minimal distance between  $i$ -th and  $j$ -th cylinders  $|\mathbf{r}_i + u(\mathbf{r}_{i+1} - \mathbf{r}_i) - \mathbf{r}_j + s(\mathbf{r}_{j+1} - \mathbf{r}_j)| \rightarrow \min$ ,

$$U_{rep} = U_{sph}^{rep} + U_{cyl}^{rep} = \sum_{ij} \varepsilon \left( \frac{\sigma_s}{r_{ij}} \right)^{12} + \sum_{ij} \varepsilon \left( \frac{\sigma_c}{|\mathbf{r}_i + u(\mathbf{r}_{i+1} - \mathbf{r}_i) - \mathbf{r}_j + s(\mathbf{r}_{j+1} - \mathbf{r}_j)|} \right)^{12} \tag{S9}$$

In Eq. (S9) constant  $\varepsilon$  is the energy scale, and  $\sigma_s$  and  $\sigma_c$  are the length scale for the spherical and cylindrical excluded volume interactions, respectively.  $U_{sph}^{rep} = 0$  when  $\sigma_s = R_{i,s} + R_{j,s}$ , where  $R_{i,s}$  and  $R_{j,s}$  are the radii of particles  $i$  and  $j$ , and  $U_{cyl}^{rep} = 0$  when  $\sigma_c = R_{i,c} + R_{j,c}$ , where  $R_{i,c}$  and  $R_{j,c}$  are the radii of cylinders  $i$  and  $j$ , and  $\varepsilon = 2.1 \times 10^5$  kJ/mol (see Table S2).

*Cell boundary*: For the elliptical cell shape, function  $\zeta_i = \left(\frac{x_i}{a}\right)^2 + \left(\frac{y_i}{b}\right)^2 + \left(\frac{z_i}{c}\right)^2$  determines whether the  $i$ -th mechanically active component (e.g. MTs, KTs and CHs) is inside the cell volume. Here  $x_i, y_i, z_i$  are the coordinates of the center-of-mass of the  $i$ -th component and  $a, b, c$  are the semi-major ( $a$ ) and the two semi-minor axes ( $b$  and  $c$ ) of the ellipse, respectively (see Table S2),

$$U_{mem} = \sum_i \frac{1}{2} \theta(\zeta_i - 1) K_{mem} r_i^2 \quad (\text{S10})$$

The repulsion potential energy (Eq. (S10)) depends on the component-boundary separation distance  $r_i$  and the boundary stiffness  $K_{mem} = 3.3 \times 10^3$  pN/nm (see Table S2); here,  $\theta(x)$  is the Heaviside step function, defined as  $\theta = 1$  for  $x = \zeta_i - 1 > 0$ , and  $\theta = 0$  otherwise.

**Dynamics of MT growth, shortening, catastrophe, and rescue:** The MT growth is described by the differential equation for MT length,

$$\frac{dl}{dt} = v_{gr} = k_{gr} \Delta l \quad (\text{S11})$$

In Eq. (S11)  $k_{gr} = 5.0 \text{ s}^{-1}$  is the growth rate and  $\Delta l = 24$  nm is the length increment. The MT shortening is described using the equation,

$$\frac{dl}{dt} = v_{sh} = -k_{sh} \Delta l \quad (\text{S12})$$

In Eq. (S12)  $k_{sh} = 18.6 \text{ s}^{-1}$  is the shortening rate (Table S1). The rate constants  $k_{gr} = 5.0 \text{ s}^{-1}$  and  $k_{sh} = 18.6 \text{ s}^{-1}$  are chosen to recover the experimental rates of MT growth and shortening,  $v_{gr} = 7.5 \text{ } \mu\text{m}/\text{min}$  and  $v_{sh} = 27 \text{ } \mu\text{m}/\text{min}$  (Table S1). The frequency of MT catastrophe is  $\omega_{cat} = 2.5 \times 10^{-3} \text{ s}^{-1}$ ; the frequency of MT rescue is  $\omega_{res} = 3.0 \times 10^{-2} \text{ s}^{-1}$  (Table S1); these are experimentally observed values of these quantities (Walker *et al.*, 1988).

**Dynamics of chromokinesin:** The walking speed of CK on the surface of MT,  $v_{CK}$ , is force-dependent and is described by the following equation

$$v_{CK} = v_0 \left(1 - \frac{f}{f_0}\right). \quad (\text{S13})$$

In Eq. (S13)  $v_0 = 1 \text{ } \mu\text{m}/\text{s}$  is the walking speed at zero force  $f$ , and  $f_0 = 50$  pN is the maximum force at which the chromokinesin bond between head (CH associated domain of CK) and legs (MT associated domain) breaks (Table S2).

**Dynamics evolution of mechanically components:** We employed Langevin Dynamics (LD) to describe the mechanical and force generating processes involving mechanical components. For a cell configuration  $\mathbf{r} = \{\mathbf{r}_i\}_{i=1}^N$  specified in terms of the positions of mechanically active components  $\mathbf{r}_i$  ( $i = 1, 2, \dots, N$ ), the cell dynamic mechanical evolution is followed by integrating the Langevin equations of motion in the overdamped limit for each mechanical component's position  $\mathbf{r}_i$ ,

$$\frac{d\mathbf{r}_i}{dt} = \frac{1}{\gamma} \frac{\partial U(\mathbf{r})}{\partial \mathbf{r}_i} + \sigma \mathbf{g}_i(t) \quad (\text{S14})$$

In Eq. (S14)  $U(\mathbf{r})$  is the total potential energy (mechanical energy function),  $F_i = \frac{\partial U(\mathbf{r})}{\partial \mathbf{r}_i}$  is the deterministic force,  $\gamma$  is the friction coefficient, and  $\mathbf{g}_i(t)$  is the Gaussian zero-average random force with the variance  $\sigma^2 = 2k_B T \gamma$  ( $T$  is the absolute temperature and  $k_B$  is the Boltzmann's constant). The Langevin equations are propagated forward in time with the timestep  $\delta t = 50$  ps at room temperature ( $T = 300$  K) using water viscosity (Table S2). For example, for the spherical particles,

$$\gamma = 6\pi\eta R_{CH} \quad (\text{S15})$$

For KT beads with  $R_{CH} = 362.5$  nm radius (Table S2), the friction coefficient is  $\gamma = 6.8 \times 10^6$  pN ps/nm. For cylindrical particles, the friction coefficient is given by  $\gamma = \frac{\gamma_{\perp} + 2\gamma_{\parallel}}{3}$ , where

$$\gamma_{\perp} = \frac{2\pi\eta l_{MT}}{\ln(l_{MT}/2R_{MT})} \quad (\text{S16})$$

$$\gamma_{\parallel} = \frac{4\pi\eta l_{MT}}{\ln(l_{MT}/2R_{MT})} \quad (\text{S17})$$

For example, for an MT cylinder of length  $l_{MT} = 1$   $\mu\text{m}$  and radius  $R_{MT} = 12$  nm (Table S2),  $\gamma = 3.3 \times 10^9$  pN ps/nm.

**Dynamics of microtubules – interactions with chromosomes:** MTs physically interact with CHs. These interactions could be categorized in the following six ways. 1) An excluded volume interaction occurs when an MT cylinder overlaps with a CH arm (or a centromere). It generates a pushing force direction oriented along the line connecting the centers of two interacting cylinders representing the MT and CH arm. The magnitude of the pushing force is determined by the parameter  $\varepsilon$  in the Lennard-Jones potential chosen so that the average pushing force is in the 10-pN range (Kliuchnikov *et al.*, 2022), to conform with the experimentally established forces (Brouhard and Hunt, 2005; Duro and Marston, 2015). 2) An MT-Ndc80 bond formation occurs when the plus-end bead of a growing MT is close enough (length of the Ndc80,  $l_{Ndc} = 65$  nm; Table S2) to a KT. The rate constant of this formation is  $k = 3.8 \times 10^9 \text{ s}^{-1}\text{M}^{-1}$  (see Table S1). At the Ndc80 density used ( $\sim 750$  Ndc80 linkers per KT; Table S2), this results in the formation of the MT-Ndc80 attachment within, on average,  $2.6 \times 10^{-3}$  s (Table S1) as soon as the MT plus-ends bumps into the KT. In the case of the limited amount of attachments, the model checks for how many MTs are already attached to the KT and disables the bond formation if this number is higher than a threshold number (3 MTs). 3) Initiation of MT catastrophe occurs right after an MT-Ndc80 bond formation. 4) Pulling by an MT takes place when the shortening MT stretches the Ndc80 spring thereby exerting the 10-pN average pulling force on the KT directed along the line connects the MT plus-end and the Ndc80 end bound to this plus-end. 5) Chromokinesin attachment can occur when a MT cylinder is close enough (length of the chromokinesin,  $l_{CK} = 50$  nm; Table S2) to a CH arm. The rate constant of this formation is  $k = 3.8 \times 10^9 \text{ s}^{-1}\text{M}^{-1}$  (see Table S1). 6) Chromokinesin walks toward the plus-end of a MT that produces a pulling force on the CH arm directed along the line connecting the MT-associated and CH-associated domains of chromokinesin.

**Numerical implementation:** The SRDDM was mapped into the *CellDynaMo* package (CUDA language), fully implemented on a GPU. In LD, the particle-particle, particle-cylinder and cylinder-cylinder interactions are the computational bottleneck. However, these interactions are described by the same empirical potential energy function. Therefore, when running Langevin Dynamics on a GPU, it is then possible to execute the same operation, e.g. generation of random forces, calculation of the potential energy, evaluation of forces, integration of Langevin equations of motion, for many particles at the same time. For the RDME, we implemented the next-subvolume method (NSM) extension (Elf and Ehrenberg, 2004) of the original Gillespie algorithm (Gillespie, 1976, 1977). In *CellDynaMo*, we implemented the multi-particle diffusion (MPD) approach to the reaction-diffusion master equation (Roberts *et al.*, 2013). Numerical routines for the generation of (pseudo)random numbers (Hybrid Taus) for RDME and LD are described in our previous publications (Zhmurov *et al.*, 2010, 2011). To



achieve top performance on a GPU, all the numerical algorithms implemented in RDME and LD have been recast into a data-parallel form so that the computational threads run the same instruction stream, but on different data sets (i.e., subcells and particles). We made the tasks compute-intensive so that, most of the time, the GPU performs computations rather than reading and writing data. These efforts enabled us to reach the biologically important timescales. For example, it takes ~72 hours of wall-clock time to generate a few ~30 min trajectories of cell dynamics on a contemporary graphics card GeForce GTX 1080.

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## Supplementary Movies

**S1 Movie. Effect of chromokinesin on final CH position and orientation:** This movie shows the first 5 min of cell dynamics and is related to Figure 6, B and D in the main text. The cell includes one CH with arms (blue); each centrosome has 1,500 MTs (green). The KT surface area is  $0.5 \mu\text{m}^2$  (orange). The pushing/pulling force per MT is 10 pN/10 pN, and the KT-MT dissociation rate depends on the phosphorylation degree of the Ndc80 linkers (see Table S2). A CH is shifted to one of the poles and the centromere axis is perpendicular to the vector connecting this pole to the centromere center (Figure 2D in the main text). The duration of the movie is 9 s (the movie is played ~30 times faster than the process).

**S2 Movie. Effect of limited number of MTs that can connect to a KT on final CH position and orientation:** This movie shows the first 5 min of cell dynamics and is related to Figure 6, B and E in the main text. The cell includes one CH with arms (blue); each centrosome has 1,500 MTs (green). The KT surface area is  $0.5 \mu\text{m}^2$  (orange). The pushing/pulling force per MT is 10 pN/10 pN, and the KT-MT dissociation rate depends on the phosphorylation degree of the Ndc80 linkers (see Table S2). The maximum of three MTs can be attached to a KT at the same time. A CH is shifted to one of the poles and the centromere axis is perpendicular to the vector connecting this pole to the centromere center (Figure 2D in the main text). The duration of the movie is 9 s (the movie is played ~30 times faster than the process).

**S3 Movie. Crowding effect:** The movie shows 15 min of cell dynamics and is related to Figure 7 in the main text. The cell includes five CH with arms (blue) and centrosomes each having 1,500 MTs (green). The KT surface area is  $0.5 \mu\text{m}^2$  (orange). The pushing/pulling force per MT is 10 pN/10 pN, and the KT-MT dissociation rate depends on the phosphorylation degree of the Ndc80 linkers (see Table S2). The maximum of three MTs can be attached to a KT at the same time. All five CHs are randomly placed and oriented within the area between the two poles. The duration of the movie is 30 s (the movie is played ~30 times faster than the process).

## Supplementary Tables

**Table S1. Chemical reactions at KT-MT interface, MT dynamic processes and transport properties of Aurora B:** Dynamic processes involve the MTs and the values of reaction rate constants associated with the MT growth, shortening, catastrophe and rescue;  $\Delta l = 24$  nm is the MT length change due to MT growth or shortening. Also listed are the biochemical processes, including enzymatic reactions (e.g. phosphorylation and dephosphorylation), association-dissociation reactions (e.g. MT associated protein Ndc80 linking MTs with KTs), and their reaction rate constants. The subscript  $p = 0, 1, \dots, 6$  denotes the number of phosphate groups attached to Ndc80 and corresponding changes in the rate constants for MT-Ndc80 complex dissociation. Also shown are the diffusion constants for Aurora B enzyme.

Chemical/dynamic process	Kinetic scheme	Rate constant $k$	Reference
MT growth	$MT_{l(gr)} \rightarrow MT_{l+\Delta l(gr)}$	$5.0 \text{ s}^{-1}$ ( $v_{gr} = 7.5 \text{ }\mu\text{m/min}$ )	(Walker <i>et al.</i> , 1988)
MT shortening	$MT_{l(sh)} \rightarrow MT_{l-\Delta l(sh)}$	$18.6 \text{ s}^{-1}$ ( $v_{sh} = 27 \text{ }\mu\text{m/min}$ )	(Walker <i>et al.</i> , 1988)
MT catastrophe	$MT_{l(gr)} \rightarrow MT_{l(sh)}$	$2.5 \times 10^{-3} \text{ s}^{-1}$	(Walker <i>et al.</i> , 1988)
MT rescue	$MT_{l(sh)} \rightarrow MT_{l(gr)}$	$3.0 \times 10^{-2} \text{ s}^{-1}$	(Walker <i>et al.</i> , 1988)
Ndc80 phosphorylation	$Ndc80_p \rightarrow Ndc80_{p+1}$	$1.5 \times 10^7 \text{ s}^{-1} \text{ M}^{-1}$	(Kliuchnikov <i>et al.</i> , 2022)
Ndc80 dephosphorylation	$Ndc80_{p+1} \rightarrow Ndc80_p$	$3.0 \times 10^7 \text{ s}^{-1} \text{ M}^{-1}$	(Kliuchnikov <i>et al.</i> , 2022)
MT-Ndc80 complex formation	$MT + Ndc80_p \rightarrow MT:Ndc80_p$	$3.8 \times 10^9 \text{ s}^{-1} \text{ M}^{-1}$	(Kliuchnikov <i>et al.</i> , 2022)
MT-Ndc80 complex dissociation	$MT:Ndc80_p \rightarrow MT + Ndc80_p$	$(1.5+0.2p) \times 10^{-3} \text{ s}^{-1}$	(Zaytsev <i>et al.</i> , 2015)
CK attachment	$MT + CK \rightarrow MT:CK$	$3.8 \times 10^9 \text{ s}^{-1} \text{ M}^{-1}$	hypothesized
CK detachment	$MT:CK \rightarrow MT + CK$	$1.5 \times 10^{-3} \text{ s}^{-1}$	hypothesized
Aurora B diffusion	$AB_v(AA_v) \rightarrow AB_{v+\xi}(AA_{v+\xi})$	$7.3 \times 10^7 \text{ nm}^2/\text{s}$	

**Table S2. Parameters used in Stochastic Reaction-Diffusion-Dynamics Model:** Numerical values of SRDDM parameters, which define: chemical kinetics and molecular transport, e.g. size of subcells and time step used in Reaction-Diffusion Master Equation based description of chemical components, dynamic cell evolution, e.g. time step, temperature, viscosity, bending rigidities, stiffness, pulling and pushing forces used in Langevin Dynamics of mechanical components, and cell morphology (shape, curvature, size, length, surface area, copy number of molecules, distance between components, etc.)

Parameter	Description	Value, units	Reference
$l_{SV}$	size of subcells in RDME	250 nm	
$\tau$	time step in RDME	$4.3 \times 10^{-5}$ s	
$\delta t$	time step in LD	50 ps	
$T$	Temperature	300 K	
$\eta$	Viscosity	1 cps	
$K_{MT,\theta}$	MT bending rigidity	$7.7 \times 10^5$ kJ/mol·rad <sup>2</sup>	(Kliuchnikov <i>et al.</i> , 2022)
$\theta_{MT,0}$	equilibrium bending angle for MT	180°	
$K_{MT,r}$	MT stretching rigidity	16.7 pN/nm	(Kliuchnikov <i>et al.</i> , 2022)
$K_{KT,r}$	stretching rigidity for sister KTs	0.83 pN/nm, 83.0 pN/nm, 333.0 pN/nm	(Kliuchnikov <i>et al.</i> , 2022)
$K_{CH,r}$	CH stretching rigidity	$3.3 \times 10^3$ pN/nm	(Kliuchnikov <i>et al.</i> , 2022)
$K_{CH,\theta}$	CH bending rigidity	$2.5 \times 10^5$ kJ/mol·rad <sup>2</sup>	(Kliuchnikov <i>et al.</i> , 2022)
$\theta_{CH,0}$	equilibrium bending angle for CH	180°	
$K_{Ndc,r}$	Ndc80 stiffness	$3.1 \times 10^2$ pN/nm	(Kliuchnikov <i>et al.</i> , 2022)
$K_{coh,r}$	stretching rigidity of cohesion ring between sister chromatids	$10^{-3}$ kJ/(mol·nm <sup>2</sup> )	(Kliuchnikov <i>et al.</i> , 2022)
$K_{mem}$	membrane stiffness	$3.3 \times 10^3$ pN/nm	(Kliuchnikov <i>et al.</i> , 2022)
$\varepsilon$	strength of repulsive potential	$2.1 \times 10^5$ kJ/mol	(Kliuchnikov <i>et al.</i> , 2022)
$f_{push}$	pushing force per MT	10 pN	
$f_{pull}$	pulling force per MT	10 pN	
$K_{CK,r}$	CK stretching rigidity	16.7 pN/nm	(Kliuchnikov <i>et al.</i> , 2022)
$v_0$	CK walking speed at zero force	1 $\mu$ m/s	(van Riel <i>et al.</i> , 2017)
$f_0$	the force at which chromokinesin dissociates from MT	50 pN	(Almeida and Maiato, 2018)
$d_{CS}$	distance between CSs (2 CS per cell)	10 $\mu$ m	(Silkworth <i>et al.</i> , 2012)
$R_{CS}$	CS bead size	0.4 $\mu$ m	(Decker <i>et al.</i> , 2011; Alieva and Uzbekov, 2016)
$N_{MT}$	number of MTs per CS	750	(McIntosh and Landis, 1971; Wollman <i>et al.</i> , 2005)
$R_{MT}$	MT bead size	12 nm	(Kononova <i>et al.</i> , 2014)
$L_{MT,max}$	The maximum length of a single cylinder fragment of MT	1 $\mu$ m	
$l_p$	MT persistence length	4 nm	(Kononova <i>et al.</i> , 2014)
$N_{KT}$	number of KTs (2 KTs per single CH)	2, 4, 6, 8, 10	
$d_{KT}$	KT-KT equilibrium distance	725 nm	(Ris and Witt, 1981)
$A_{KT}$	KT surface area	0.03 $\mu$ m <sup>2</sup> , 0.15 $\mu$ m <sup>2</sup> , 0.36 $\mu$ m <sup>2</sup>	(Drpic <i>et al.</i> , 2018)
$\chi$	KT surface curvature	0 – 1	
$R_{CH}$	size of beads representing KT and CH	362.5 nm	(Ris and Witt, 1981)
$l_{ch}$	CH contour length	varied	
$N_{Ndc}$	number of Ndc80 per KT surface	750	
$l_{Ndc}$	size of Ndc80-complex	65 nm	(Wei <i>et al.</i> , 2005)
$R_{Ndc}$	size of Ndc80 KT-associated domain	4 nm	(Ciferri <i>et al.</i> , 2008)
$l_{CK}$	length of CK (1 CK per MT)	50 nm	(Subramanian <i>et al.</i> , 2013)
$R_{CK}$	size of MT- and CH-associated domains of CK	4 nm	
$R_A$	size of Aurora B ( $\sim 2.0 \times 10^3$ per CH)	2.9 nm	(Elkins <i>et al.</i> , 2012)
P:A ratio	Phosphatase to Aurora B ratio ( $\sim 10$ per KT surface)	0.1	(Kliuchnikov <i>et al.</i> , 2022)
$a$	ellipsoidal shape: $a$ is semi-major axis, $b$ , $c$ are semi-minor axes;	8 $\mu$ m	
$b, c$	rectangular shape: $a$ , $b$ , and $c$ are length, width, and height	5 $\mu$ m, 5 $\mu$ m	