doi: 10.1038/nature05786

Experimental and Theoretical Study of Mitotic Spindle Orientation

Manuel Théry[1,2*], Andrea Jiménez-Dalmaroni[3*], Victor Racine[1], Michel Bornens[1†] and Frank Jülicher[3†]

[1]*Institut Curie, CNRS UMR144, Compartimentation et Dynamique Cellulaire, 26 rue d'Ulm 75248, Paris, France.*

[2]*Commissariat à l'Energie Atomique, DSV, iRTSV, Laboratoire Biopuces, 17 rue des Martyrs 38054, Grenoble, France.*

[3]*Max Planck Institute for the Physics of Complex Systems, Nöthnitzer Str. 38, 01187 Dresden, Germany.*

[*]*These authors contributed equally to this work.*

[†]Corresponding authors

Supplementary Information

Supplementary Text

In our two-dimensional representation, the cell is characterized by a circle with radius R (Fig. 1c,d). The spindle geometry is described by two poles, arranged symmetrically with respect to the cell centre, with a separation $2a$. Astral microtubules radiate from these poles with a uniform distribution within a finite angular interval chosen such that microtubules radiating from each pole reach half of the circular perimeter. The orientation of this spindle geometry is described by the angle ϕ between the spindle axis and the x-axis (Fig. 1c). Each point $\vec{R}$ on the cell cortex is described by a cortical angle ψ as

$$\vec{R}(\psi) = R\begin{pmatrix}\cos\psi\\ \sin\psi\end{pmatrix}. \qquad \text{(S1)}$$

We introduce the unit vector

$$\vec{\mathrm{m}}(\psi,\phi) = \begin{pmatrix}\cos\gamma(\psi,\phi)\\ \sin\gamma(\psi,\phi)\end{pmatrix}, \qquad \text{(S2)}$$

that points in the direction of the astral microtubules that reach the cortex at the angle ψ, for a given spindle orientation ϕ. Here $\gamma=\alpha(\theta)+\phi$ is the angle between the x-axis and the local microtubule orientation (Fig. 1c). The angle α is a function of $\theta=\psi-\phi$. Cortical forces exerted per angular element on the spindle in a direction tangential to the astral microtubules are given by

$$\vec{\mathrm{f}}(\psi,\phi) = \mathrm{F}(\psi)\,\rho_{MT}(\psi-\phi)\,\vec{\mathrm{m}}(\psi,\phi). \qquad \text{(S3)}$$

Here $F(\psi)$ denotes the magnitude of the force acting per microtubule at the cortical angle ψ, and $\rho_{MT}(\theta)$ is the angular density of microtubules reaching the cell cortex at an angle θ relative to the spindle axis:

$$\rho_{MT}(\theta) = \frac{N_{MT}}{2\pi}\frac{\mathrm{d}\alpha}{\mathrm{d}\theta}\ . \qquad \text{(S4)}$$

Here, N_{MT} is the total number of microtubules emerging from one spindle pole in the planar projection. The total force exerted by the force generators on the spindle acts to displace the spindle off the centre of the rounded cell until it is balanced by a centring force due to microtubule compression and buckling[1]. As shown in supplementary Fig. S2, spindle displacements in HeLa cells are very small and can be neglected. However, the force generators also exert a net torque which is given by

$$\tau_z(\phi) = \int_{-\pi}^{\pi} d\psi\,(R_x f_y - R_y f_x)\,, \qquad \text{(S5)}$$

where the vector $\vec{R}(\psi)$, with components $\mathrm{R_x}$ and $\mathrm{R_y}$, points from the cell centre to the cortical position with angle ψ (Fig. 1c). Note that in equation (2) in the main text the vectors $\vec{R}(\psi)$

and $\vec{f}(\psi,\phi)$ have an additional z-component which is zero in order to permit a condensed notation. The torque τ_z depends only on the spindle orientation ϕ. It is convenient to define the effective energy landscape

$$W(\phi) = -\int_{-\pi/2}^{\phi} \tau_z(\phi')\,\mathrm{d}\phi'. \qquad \text{(S6)}$$

Stable spindle orientations thus correspond to minima of the potential $W(\phi)$. Our key assumption, that retraction fibres locally activate cortical force generators, can be written in the form

$$\mathrm{F}(\psi) \propto \rho_r(\psi), \qquad \text{(S7)}$$

in which $\rho_r(\psi)$ denotes the angular density of retraction fibres reaching the cortex radially at angle ψ. The angular distribution $\rho_r(\psi)$ of retraction fibres depends on the pattern geometry. It can be estimated using the contour line of the pattern shape along which retraction fibre attach (indicated by a red pattern outline in Fig. 1d and in Figs. 2-4) and the position of the cell centre on the pattern. We parameterised the shape of this adhesive boundary line (shown as red outlines in Figs. 1-4) by the positions $(x(\psi),y(\psi))$ measured from the cell centre. The local density of retraction fibres at the cell cortex $\rho_r(\psi) \propto \mathrm{g}(\psi)$ is then proportional to the dimensionless quantity

$$\mathrm{g}(\psi) = \frac{1}{\mathrm{R}}\left[\left(\frac{\mathrm{d}x}{\mathrm{d}\psi}\right)^2 + \left(\frac{\mathrm{d}y}{\mathrm{d}\psi}\right)^2\right]^{1/2}. \qquad \text{(S8)}$$

Therefore, we can express the force per microtubule as

$$\mathrm{F}(\psi) = C\,\mathrm{g}(\psi), \qquad \text{(S9)}$$

in which we have introduced the coefficient C that has units of force. It characterizes the activation of force generators by the density of retraction fibres. With these definitions, we can calculate the angular torque $\tau_z(\phi)$ for any given pattern geometry, using the centre of mass of the pattern as an estimate for the average position of the cell centre on the pattern (see supplemental Fig. S1). Note that the cortical forces, which determine spindle orientation, are purely internal to the cell, while the cell position is determined by the balance of external forces which are not the subject of this work.

In order to determine the distribution of spindle orientations, we write a dynamic equation for the spindle orientation angle

$$\eta\frac{\mathrm{d}\phi}{\mathrm{d}t} = \tau_z(\phi) + \xi(t). \qquad \text{(S10)}$$

Here η is an effective friction coefficient for spindle rotations, and $\xi(t)$ is a random torque with zero average that is added to the average torque τ and accounts for the effects of fluctuations in the system. Using for simplicity a Gaussian white noise with variance

$$\langle \xi(t)\xi(t') \rangle = 2D\eta\delta(t-t') \,, \tag{S11}$$

and noise strength D, the orientation ϕ reaches for long times a stationary angular distribution

$$P(\phi) = N \exp\left(-\frac{W(\phi)}{D}\right), \tag{S12}$$

Here N is a normalization factor. In order to compare these calculated angular distributions with experiments, we introduce the dimensionless energy profile,

$$w(\phi) = \frac{2\pi}{C\, N_{MT}\, \mathrm{R}} W(\phi), \tag{S13}$$

that only depends on the pattern geometry and the ratio a/R. We can write

$$P(\phi) = N \exp\left(-w(\phi)/d\right), \tag{S14}$$

in which

$$d = \frac{2\,\pi\, D}{C\, N_{MT}\, \mathrm{R}} \tag{S15}$$

is a dimensionless coefficient which combines the effects of many unknown details. These include the noise strength, the friction coefficient, as well as the strength of the coupling of retraction fibres to the activity of force generators and the number of force generators. The latter two are both described by the parameter C.

Supplementary Discussion

Our model did not account quantitatively for the exact points at which transitions of orientation angles occurred when the H-shaped pattern was continuously deformed. However, these transitions result from a subtle balance of opposing torques that can be captured qualitatively in our very simple description. Similarly, to describe the delicate balance that governs the transition of the preferred spindle orientations between the arrow- and the crossbow-shaped patterns, the adhesive pattern outlines used in the calculations was slightly adjusted, in accordance with the experimentally observed retraction fibre distributions (Fig. 4). Close to such transitions of spindle orientation, details of the retraction fibre distributions and of the molecular processes governing activation of force generators become important for a fully quantitative description. To incorporate such details requires a refined analysis of the distribution of retraction fibres. It could also involve nonlinear corrections to our simple linear relation between cortical pulling force and the density of retraction fibres.

Our model is based on the hypothesis that the activity of cortical force generators depends on the local density of retraction fibres. How this is achieved needs to be investigated[2]. The activation as well as the recruitment of cortical force generators could be associated with well characterised cortical cues such as the presence of complexes associated to heterotrimeric G proteins known to regulate spindle orientation[2-8]. In vivo, any source of cortical heterogeneity, either due to external chemical gradients or non homogeneous spatial distribution of cell contacts, that leads to a differential activation or recruitement of cortical force generators could guide spindle orientation according to our theoretical model.

Supplementary Notes

This work was supported by a grant from the Human Frontier Science Program, ref RGP0064/2004.

1. Grill, S. W., Kruse, K. & Julicher, F. Theory of mitotic spindle oscillations. *Phys Rev Lett* **94**, 108104 (2005).
2. Thery, M. & Bornens, M. Cell shape and cell division. *Curr Opin Cell Biol* **18**, 648-57 (2006).
3. Du, Q. & Macara, I. G. Mammalian Pins is a conformational switch that links NuMA to heterotrimeric G proteins. *Cell* **119**, 503-16 (2004).
4. Izumi, Y., Ohta, N., Hisata, K., Raabe, T. & Matsuzaki, F. Drosophila Pins-binding protein Mud regulates spindle-polarity coupling and centrosome organization. *Nat Cell Biol* **8**, 586-93 (2006).
5. Sanada, K. & Tsai, L. H. G protein betagamma subunits and AGS3 control spindle orientation and asymmetric cell fate of cerebral cortical progenitors. *Cell* **122**, 119-31 (2005).
6. Colombo, K. et al. Translation of polarity cues into asymmetric spindle positioning in Caenorhabditis elegans embryos. *Science* **300**, 1957-61 (2003).
7. Lechler, T. & Fuchs, E. Asymmetric cell divisions promote stratification and differentiation of mammalian skin. *Nature* **437**, 275-80 (2005).
8. Yamashita, Y. M., Jones, D. L. & Fuller, M. T. Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome. *Science* **301**, 1547-50 (2003).

Supplementary Figures and legends

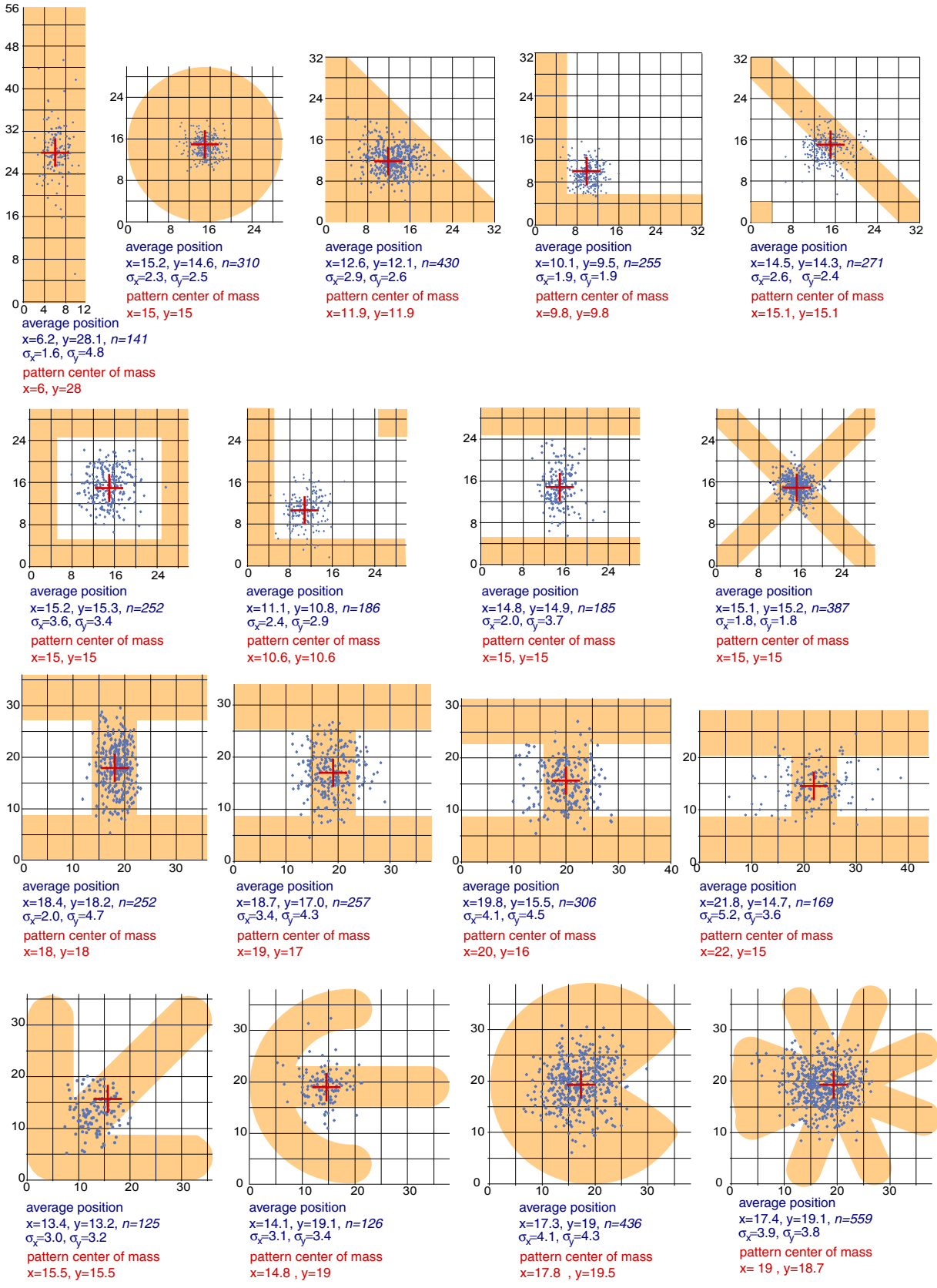

Figure S1. Positions of cell centres on different patterns.
Experimentally observed positions of the centres of mitotic cells (blue dots) on the different micro-patterns (orange). Cell centres are clustered near the geometric centre the patterns (red cross). This geometric centre is calculated as the centre of mass assuming a constant surface mass density on the pattern. The positions of the centre of mass, the average position of cell centres and the standard deviations of the distributions are indicated.

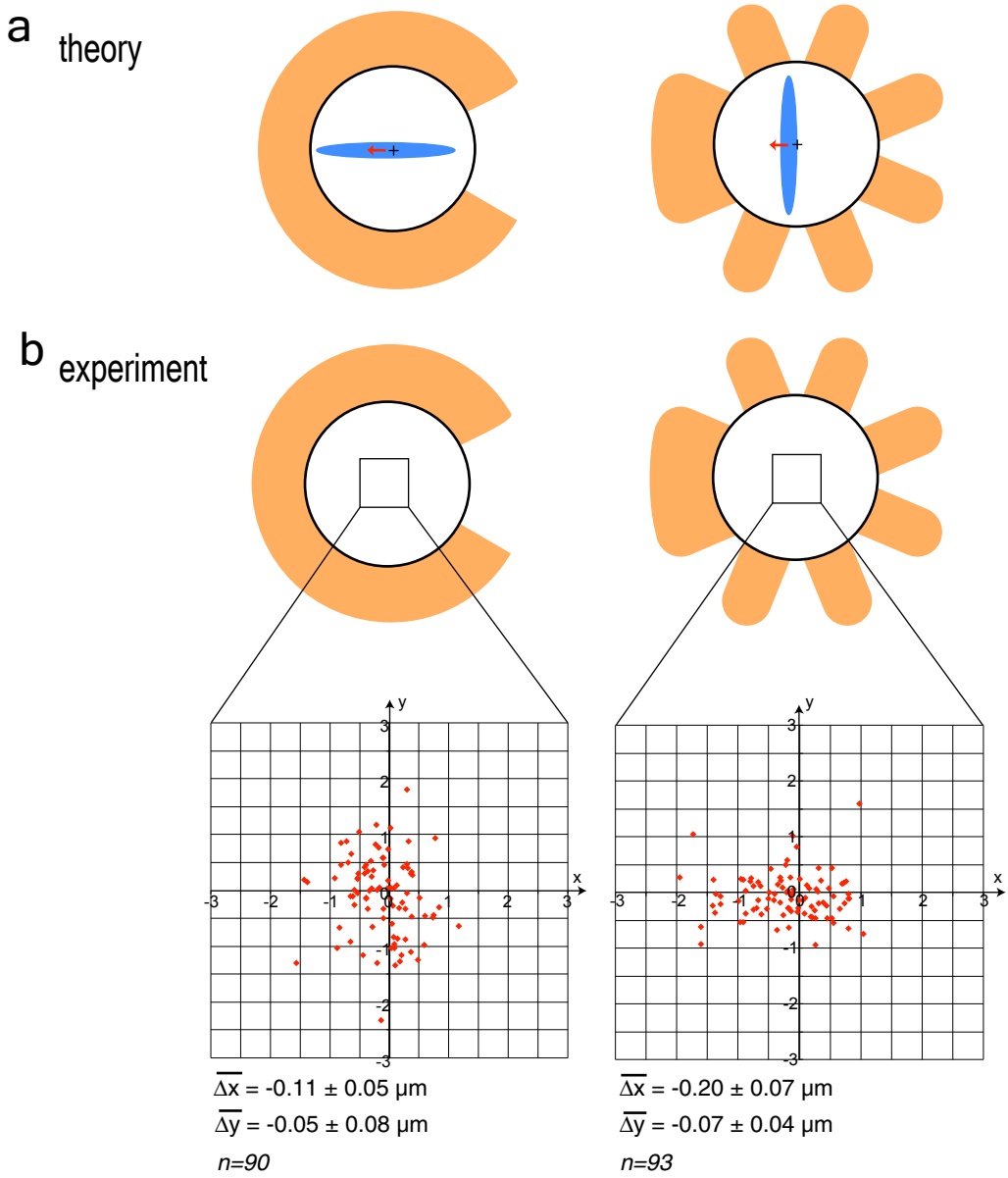

Figure S2. Metaphase plate displacements with respect to the cell centre, determined for symmetric and asymmetric spindle orientations

a, The net force exerted by cortical force generators can induce spindle displacements. Such spindle displacements can lead to shifts in the position of the metaphase plate in a direction of the average cortical force. We calculated the direction of the average force for two pattern shapes (orange). The orientation of the force vector is shown (red arrow) to indicate the direction of the expected average displacement of the metaphase plate (blue bar).

b, The positions of the centre of the cell (determined by a phalloidin staining of the cortex), and of the metaphase plate (determined by Hoechst staining of DNA), were determined, using an automated threshold and morphometric image analysis. The measured positions of the metaphase plates with respect to the cell centre (at the origin of the x-y coordinate system) are shown for two different patterns (red dots). Distances are given in microns. The average displacements and in x and y directions and the standard deviations of displacements are indicated for both patterns. Average displacements were small (less than 200nm) compared to the cell radius (10μm), which shows that spindle displacements can be neglected in our theoretical analysis. Note that the metaphase plate is displaced on average in the negative x-direction, consistent with the direction of the average total force calculated in our model (see a).

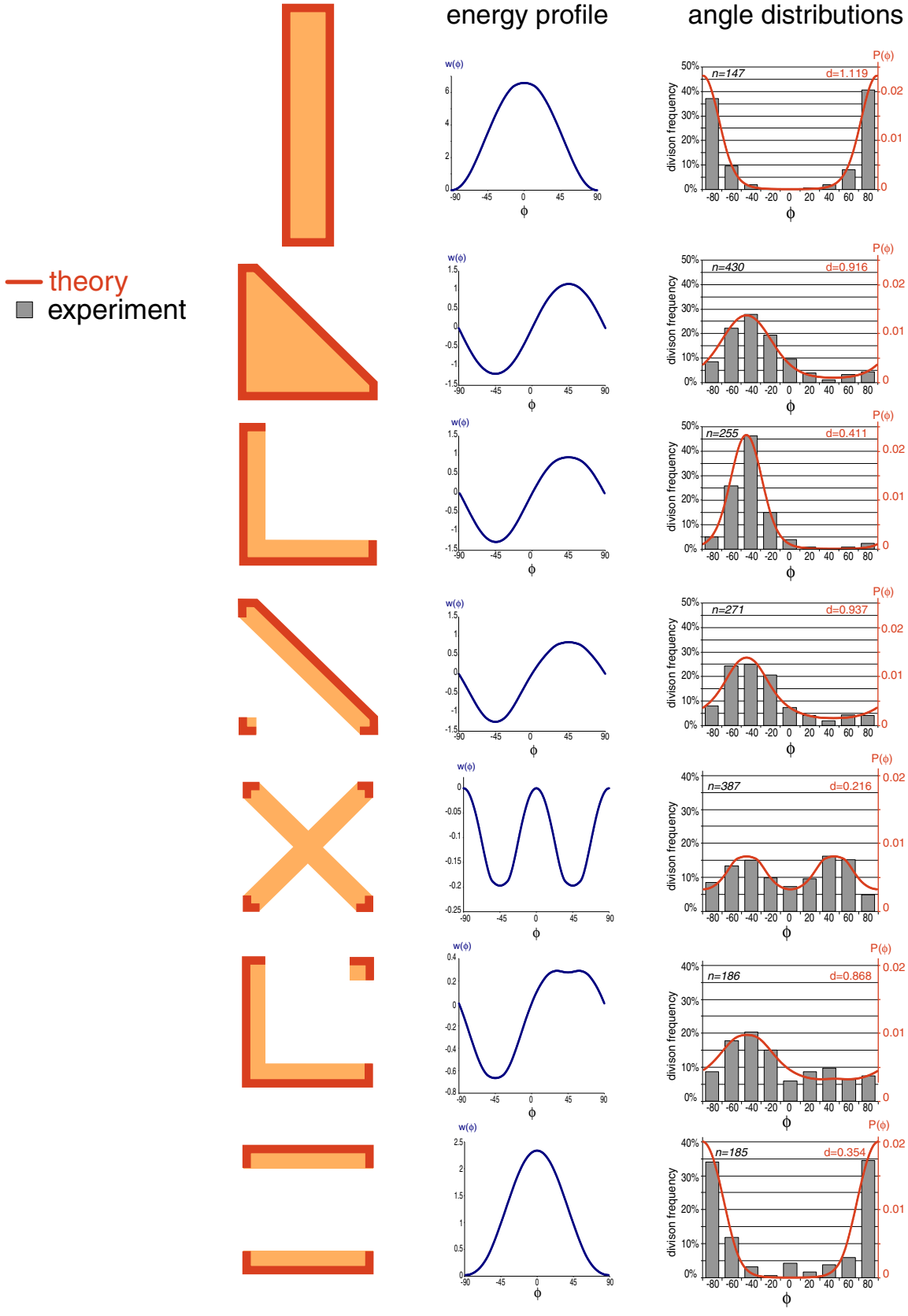

Figure S3. Spindle orientation on various pattern geometries

Our theory can quantitatively account for the observed spindle orientation angles on many different patterns studied experimentally. For each pattern geometry (orange), the convex outline of the pattern (which corresponds to the line of attachment of retraction fibres) is indicated (red). Based on this geometry, we calculate the energy profiles $w(\phi)$ (blue) that characterized the spindle orientations. The angular probability density $P(\phi)=N \exp(-w(\phi)/d)$ (red curve) is related to the potential profile by the parameter d. We fit the calculated angular distribution to the experimentally measured angular histogram using d as a single fit parameter. The number n of cells used to obtain the histograms and the best fit values of d are indicated for each pattern.