SUPPLEMENTAL MATERIALS

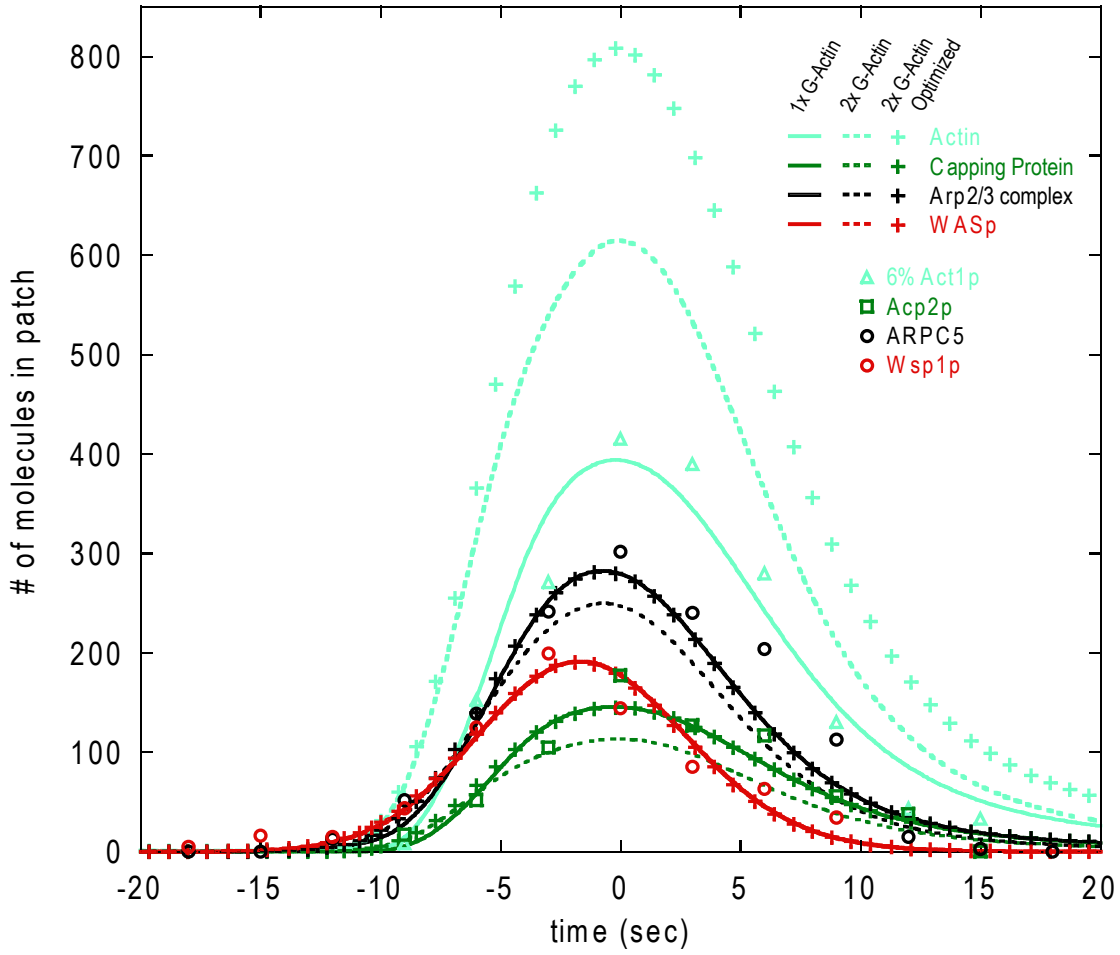
Supplemental Figures

Figure S1: Simulation of the main model with the total actin concentration two times higher than measured in heterozygous diploid cells in Sirotkin et al. (2010). The plain lines are the output of the main model with 21.6 µM total actin as estimated in the diploid *S. pombe* in Sirotkin et al. (2010). The dashed lines are the output of the model for 43.2 µM total actin and the parameters from the main model in Table 1. The crosses are after optimization of $k_{Chop} = 1.4\ 10^{-3}\ \mu M^{-1}s^{-1}$ and $k^{+}_{Cap} = 6.8\ \mu M^{-1}s^{-1}$. The color code is the same as in Figure 2: simulated WASp (red curves and +); measured Wsp1p (red circles); simulated Arp2/3 complex (black curve and +); measured ARPC5 (black circles); simulated 6% of polymerized actin subunits (teal curve and +); measured 6% of Act1p (teal triangles); simulated capping protein (green curve and +); and measured Acp2p (green squares).

Figure S2: Sensitivity analysis of the model. The model was simulated with each parameter varied ± one order of magnitude to determine the sensitivity of the time course of patch proteins on parameter values. (A-D) Dependence of times of protein peaks in patches on parameter values. (E-H) Dependence of the amplitudes of protein peaks in patches on parameter values. (I-L) Dependence of the width of the protein time courses in patches on parameter values. The width is defined as the difference between the times when the signal reaches 25% of its peak value during the assembly and during the disassembly. (A, E, I) Actin; (B, F, J) Capping protein; (C, G, K) Arp2/3 complex; (D, H, L) WASp. Color code for varied parameters: yellow dashed line, $k^{+}_{WASpGBinding}$; orange dotted line, $k^{+}_{ArpComplexFormation}$; red dotted and dashed line, $k^{+}_{ARPGWBindingF}$; brown plain line, $k^{+}_{ArpActivation}$; dark blue plain line, $k^{+}_{Polymerisation}$; blue dashed line, k^{+}_{Cap}; light blue dotted line, $k_{Hydrolysis}$; light blue dotted and dashed line, $k^{+}_{COFBinding}$; plain light green line, k_{Chop}. Parameters for the reverse reactions are not shown, since varying these parameters has little effect on the time courses of proteins in patches.

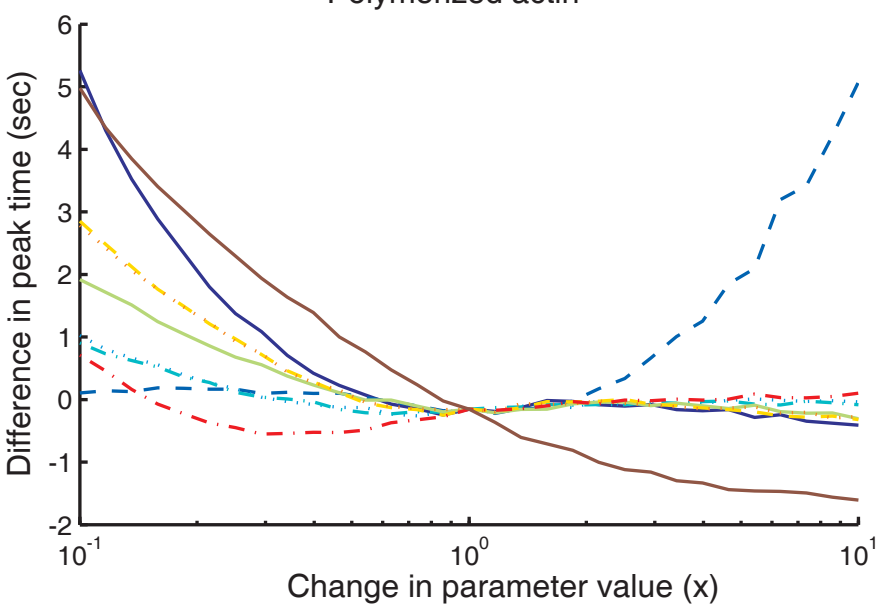
Figure S3: Two-dimensional parameter scan to find pairs of values that give simulations with good fits to the experimental data. Each parameter was varied ± one order of magnitude with the value on the axis of the graph representing the ratio of the tested value of parameter to its value in the main model of this paper. Each dot represents a pair of parameter values producing simulated time courses for total actin, total WASp, total Arp2/3 complex and capping protein within the following fitness limit: the square root of the sum of the residuals for all 3 outputs is less than 2 times the sum of the residuals for the same species in the main model. The color code gives the ratio between the sums of these residuals for the modified parameters and the main model. Gray crosses are the pairs of parameters in the main model. The figures show the balance between the influences of pairs of parameters. (A) $k^{+}_{Polymerisation}$ and k^{+}_{Cap}. (B) k_{Chop} and $k^{+}_{COFBinding}$. (C) $k^{+}_{COFBinding}$ and $k_{Hydrolysis}$. (D) $k_{Hydrolysis}$ and k_{Chop}. (E) $k^{+}_{WaspGBinding}$ and $k^{+}_{ArpComplexFormation}$.

Figure S4: Schema of a small volume expanding with the growth of an actin network around a sphere (A) or a cylinder (B).

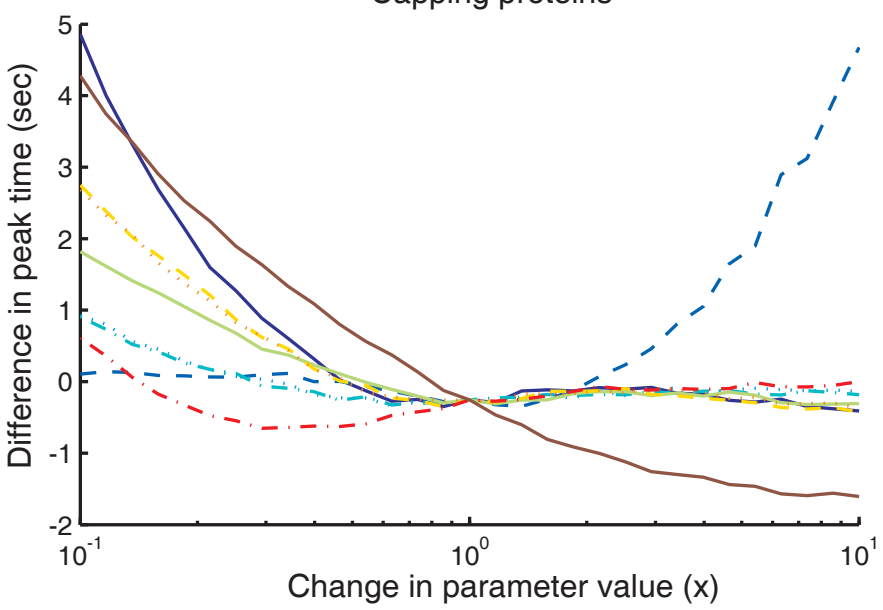


Supplemental Figure 1

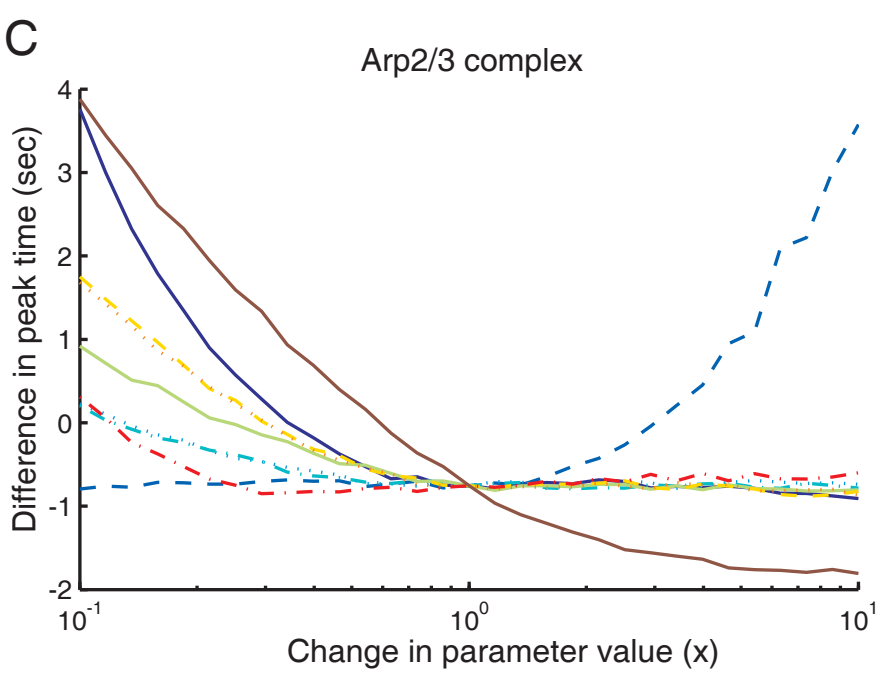
A


Polymerized actin
Difference in peak time (sec)
Change in parameter value (x)

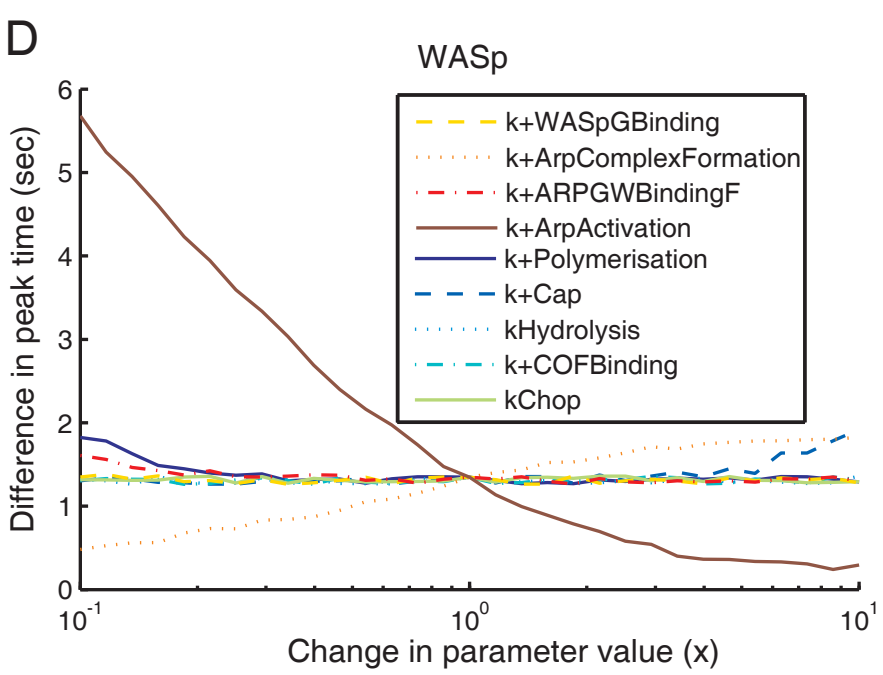
B


Capping proteins
Difference in peak time (sec)
Change in parameter value (x)

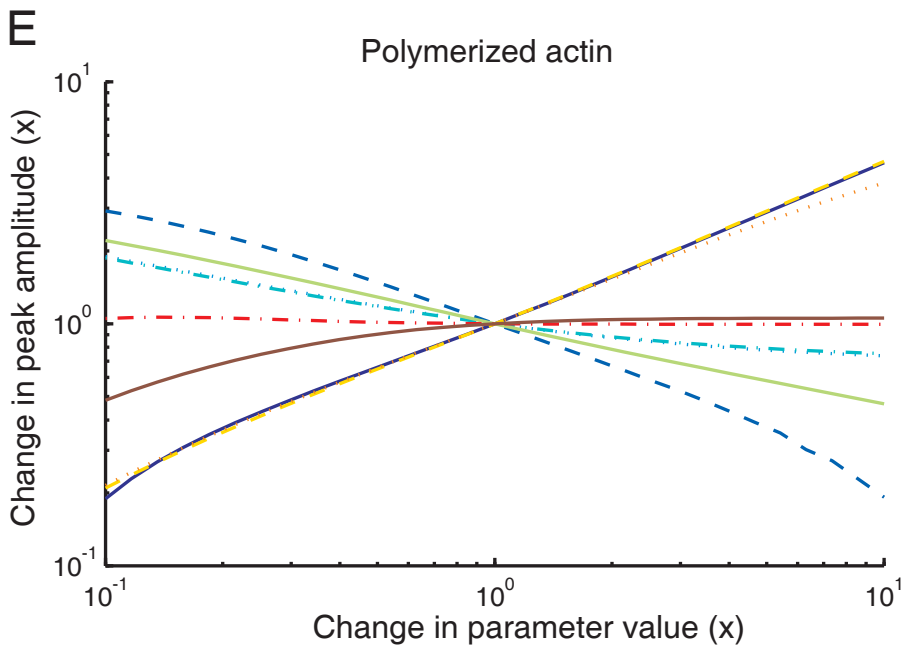
C


Arp2/3 complex
Difference in peak time (sec)
Change in parameter value (x)

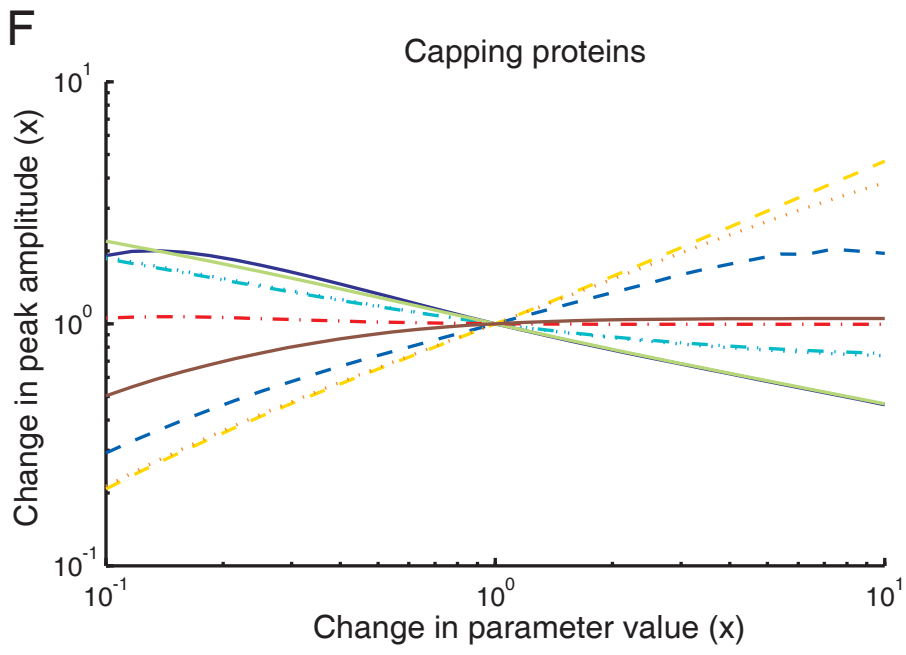
D


WASp
k+WASpGBinding
k+ArpComplexFormation
k+ARPGWBindingF
k+ArpActivation
k+Polymerisation
k+Cap
kHydrolysis
k+COFBinding
kChop
Difference in peak time (sec)
Change in parameter value (x)

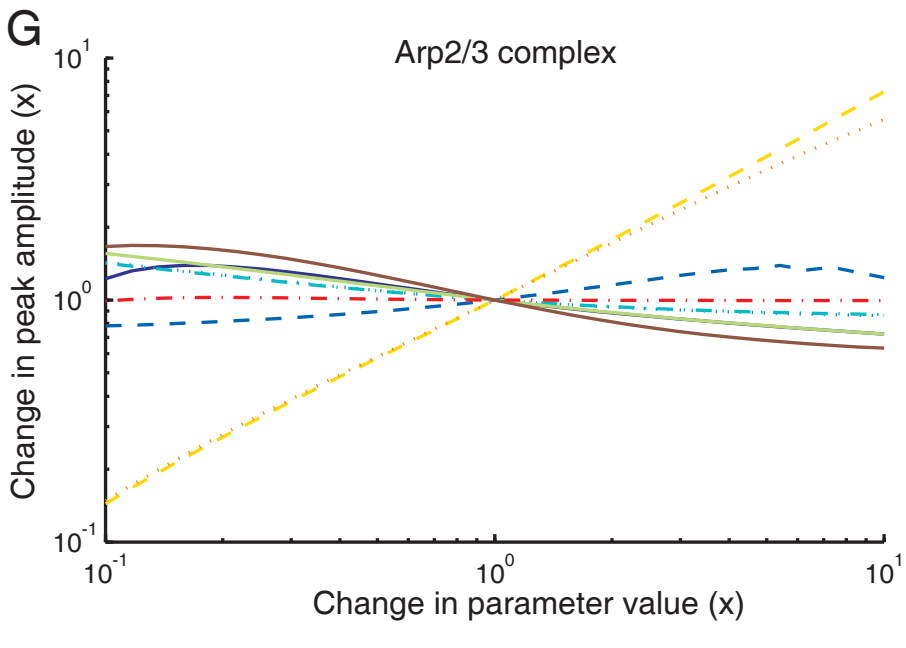
E


Polymerized actin
Change in peak amplitude (x)
Change in parameter value (x)

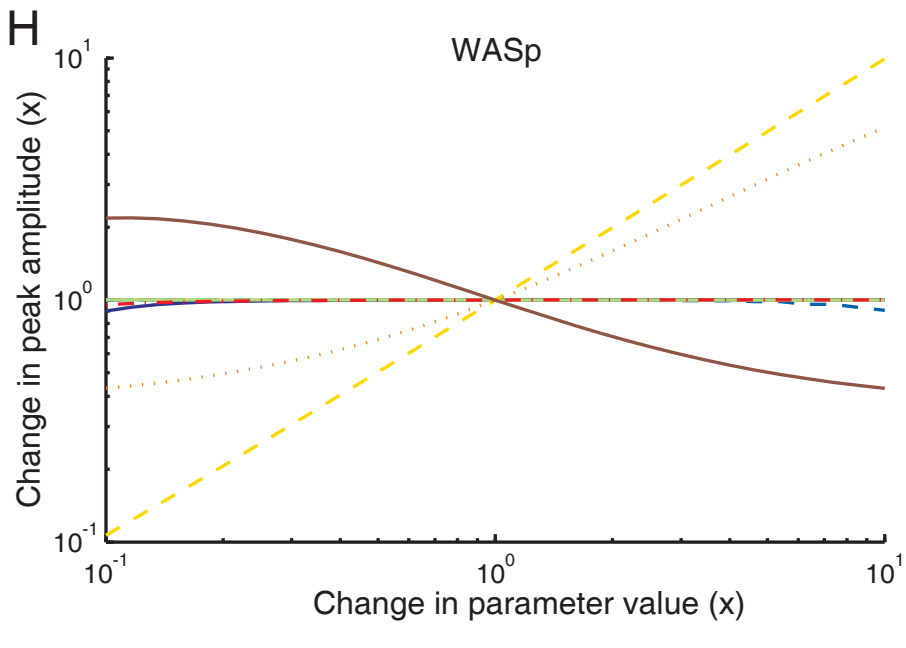
F

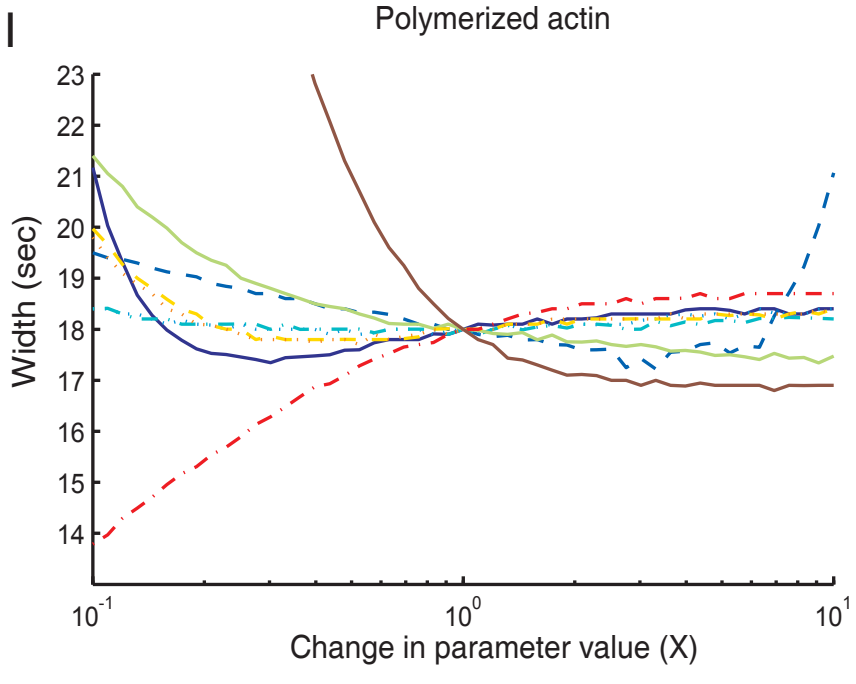

Capping proteins
Change in peak amplitude (x)
Change in parameter value (x)

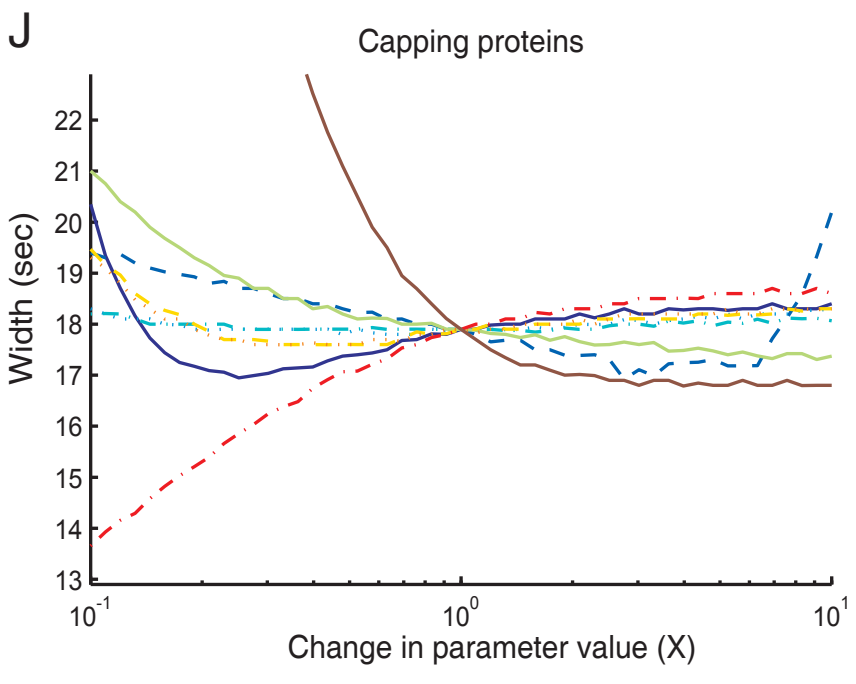
G

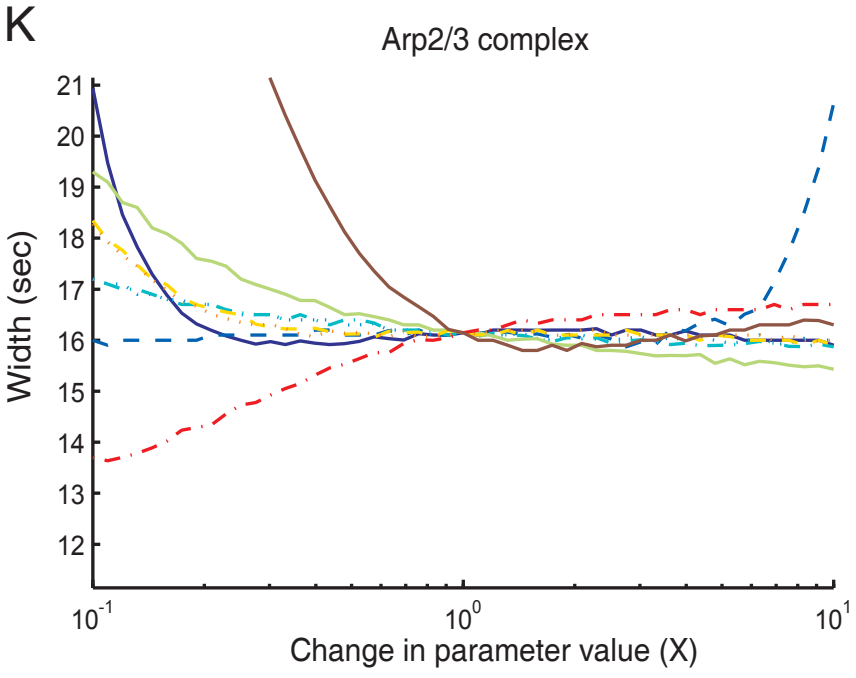

Arp2/3 complex
Change in peak amplitude (x)
Change in parameter value (x)

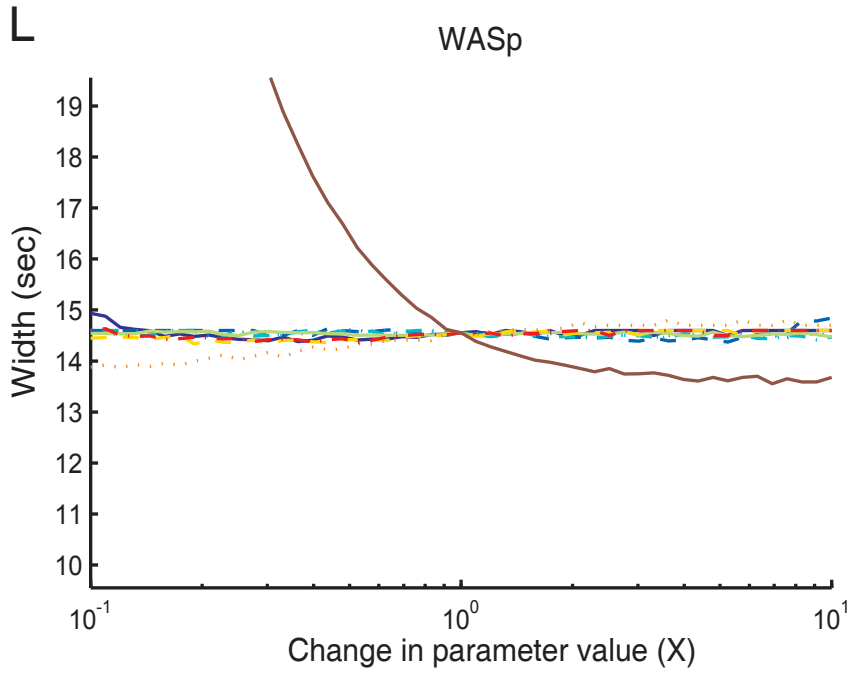
H


WASp
Change in peak amplitude (x)
Change in parameter value (x)

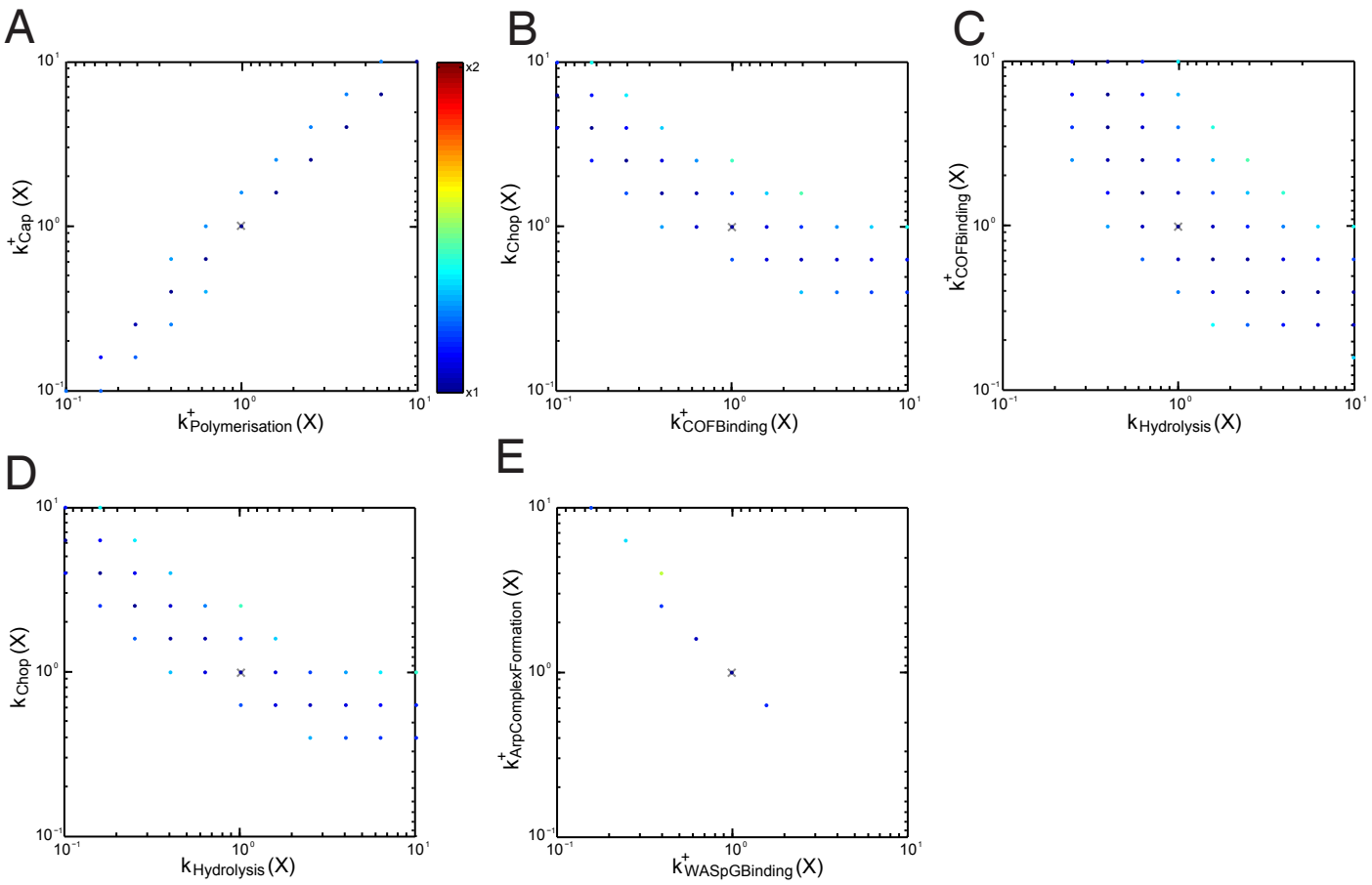




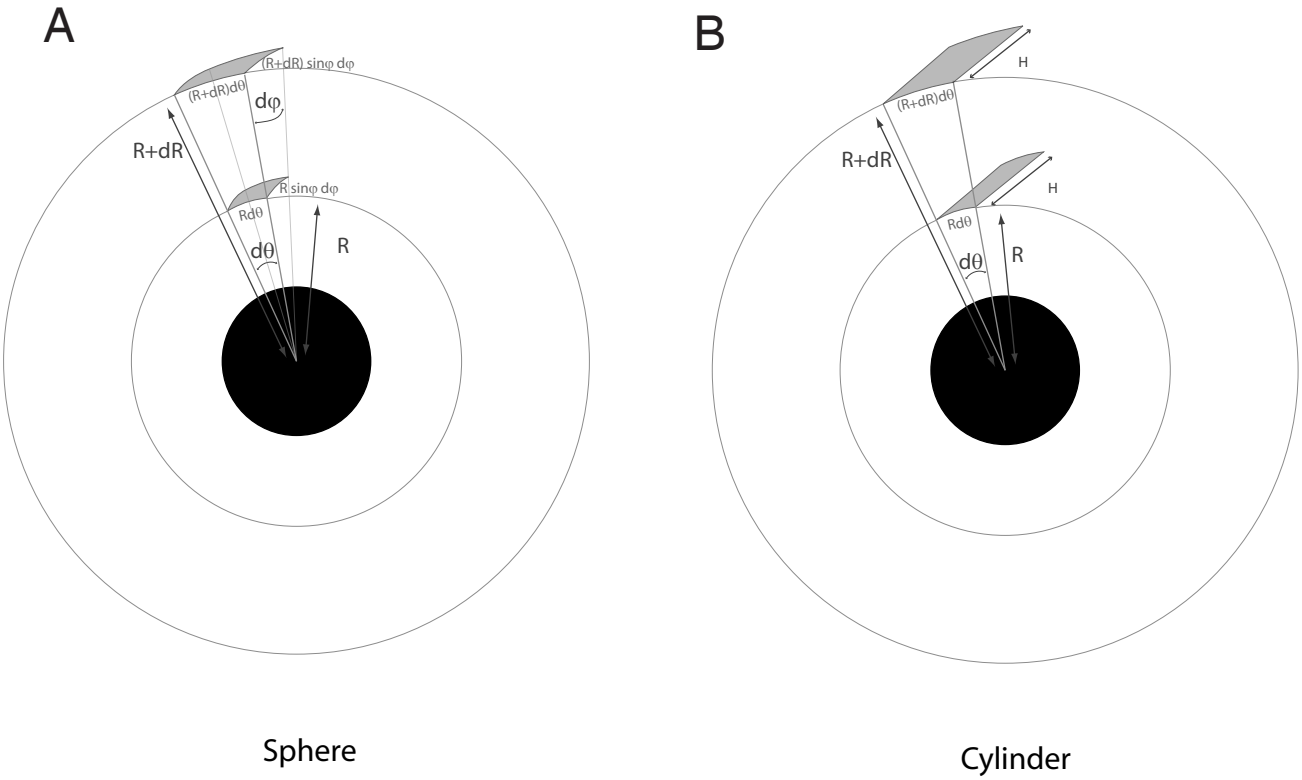




Supplemental Figure 2



Supplemental Figure 3



Sphere

Cylinder

Supplemental Figure 4

Supplemental Methods

Concentration gradient due to actin gel expansion

We consider a network of actin expanding from the surface of a vesicle, considered as a sphere, or from a tubule, considered as a cylinder. We assume that all the barbed ends are directed towards the surface and that polymerization pushes the actin network radially away from the surface as it expands tangentially. This creates a concentration gradient of actin filaments with the highest concentration near the surface and the lowest concentration at the periphery. Due to symmetry, the actin surface density C(R) and the actin concentration will be the same at a given distance R from the center of the sphere or the axis of the cylinder.

Expansion around a sphere

Consider a surface at a radial distance R with an inclination angle dθ and an azimuth angle d (Figure S4A). Polymerization pushes this volume radially away from the membrane at distance R+dR and expands tangentially to (R+dR)dθ and (R+dR)sin(φ)dφ
Thus, on the surface of a sphere at distance R, there are

$$N(R) = \int_0^{2\pi} \int_0^{\pi} C(R) \times \mathcal{N}_A \times (Rd\theta) \times (R\,sin(\varphi)\,d\varphi) = 4\pi\mathcal{N}_A R^2 C(R)$$

actin molecules, where C(R) is the surface density of actin filaments at distance R and $\mathcal{N}_A$ is the Avogadro's constant.
At distance R+dR, there are $N(R+dR) = 4\pi\mathcal{N}_A(R+dR)^2 C(R+dR)$ molecules.
Because polymerized actin is conserved on the surface of the expanding sphere, the number of molecules is the same on the surfaces of the spheres of radius R and R+dR. Thus, we have
$N(R) = N(R+dR) = N_0$

$$C(R+dR) - C(R) = \frac{N_0}{4\pi\mathcal{N}_A}\left(\frac{1}{(R+dR)^2} - \frac{1}{R^2}\right) = \frac{C_0}{R^2}\left(\frac{1}{\left(1+\frac{dR}{R}\right)^2} - 1\right)$$

with $C_0 = \frac{N_0}{4\pi\mathcal{N}_A}$.

If dR is very small compared to R, then we have $C(R+dR) - C(R) = -2\frac{C_0}{R^3}dR$

Thus, $\frac{dC(R)}{dR} = -2\frac{C_0}{R^3}$ and $C(R) = \frac{C_0}{R^2} + K$, with K being a constant. It is reasonable to assume that the concentration of actin filaments outside of the patch is null, so if we consider that the patch radius is R_{max}, we have:

$$C(R) = C_0\left(\frac{1}{R^2} - \frac{1}{{R_{max}}^2}\right)$$

Given spherical symmetry, the total number of molecules in the patch, i.e. between the radius R_{min} and R_{max} is:

$$\#\ Factin = \int_{R_{min}}^{R_{max}} C(R) \times \mathcal{N}_A \times 4\,\pi R^2 dR$$

$$\#\ Factin = \frac{4}{3{R_{max}}^2}\pi \mathcal{N}_A C_0 \left(2{R_{max}}^3 - 3R_{min}{R_{max}}^2 - {R_{min}}^3\right)$$

If we consider that the density of actin filaments is homogeneous in the patch with a concentration C_{Mean}, as we do in our main model, then the total number of polymerized actin molecules is:

$$\#\ Factin = C_{Mean}\mathcal{N}_A \frac{4}{3}\pi\left({R_{max}}^3 - {R_{min}}^3\right)$$

Thus, we have $C_0 = C_{Mean}\frac{\left({R_{max}}^3 - {R_{min}}^3\right){R_{max}}^2}{2{R_{max}}^3 - 3R_{min}{R_{max}}^2 - {R_{min}}^3}$

Finally, the concentration of F-actin at the surface of the sphere is:

$$C(R_{min}) = C_{Mean}\frac{\left(\left(\frac{R_{max}}{R_{min}}\right)^3 - 1\right)}{2\left(\frac{R_{max}}{R_{min}}\right)^3 - 3\left(\frac{R_{max}}{R_{min}}\right)^2 - 1}\left(\left(\frac{R_{max}}{R_{min}}\right)^2 - 1\right)$$

For a vesicle of diameter 50 nm inside an actin patch of diameter 300 nm, the concentration of polymerized actin is 23.3 fold higher at the surface of the vesicle than estimated for a homogeneous patch.

Expansion around a cylinder

Now we consider a surface at a radial distance R from the axis in the middle of a tubule, with an inclination angle dθ and a depth H (Figure S4B). Actin polymerization pushes this volume radially away from the membrane at distance R+dR and the volume expands tangentially to (R+dR)dθ. Since the tubule is a cylinder, there is no expansion in depth so H is constant. Thus, at the surface the cylinder with radius R, there are

$$N(R) = \int_0^{2\pi}\int_0^{\pi} C(R) \times \mathcal{N}_A \times H \times (Rd\theta) = 4\pi\mathcal{N}_A RHC(R)$$

molecules, where C(R) is the surface density of F-actin at distance R.
At distance R+dR, there are $N(R+dR) = 4\pi\mathcal{N}_A(R+dR)HC(R+dR)$ molecules.

Because polymerized actin is conserved on the surface of the expanding cylinder, the number of molecules is the same in the volumes at distances R and R+dR. Thus, we have $N(R) = N(R + dR) = N_0$

$$C(R + dR) - C(R) = \frac{C_0}{R}\left(\frac{1}{1 + \frac{dR}{R}} - 1\right)$$

with $C_0 = \frac{N_0}{4\pi \mathcal{N}_A RH}$.

As in the case of the sphere, after a 1st degree Taylor expansion, integration and assuming that the concentration is null at R_{max}, we have:

$$C(R) = C_0\left(\frac{1}{R} - \frac{1}{R_{max}}\right)$$

Given cylindrical symmetry, the total number of polymerized actin molecules is:

$$\#\ Factin = \int_{R_{min}}^{R_{max}} C(R) \times \mathcal{N}_A \times 2\,\pi R H dR$$

$$\#\ Factin = \frac{\pi H \mathcal{N}_A C_0}{2R_{max}}\ (R_{max} - R_{min})^2$$

If we consider now that the actin is homogeneous around the tubule with a concentration C_{Mean}, as we do in our main model, then the total number of polymerized actin molecules is:

$$\#\ Factin = C_{Mean}\mathcal{N}_A\pi\left({R_{max}}^2 - {R_{min}}^2\right)H$$

Thus, we have $C_0 = C_{Mean}\frac{2R_{max}(R_{max}+R_{min})}{(R_{max}-R_{min})}$

Finally, the concentration of polymerized actin at the surface of the cylinder is:

$$C(R_{min}) = C_{Mean}\frac{R_{max} + R_{min}}{R_{min}}$$

For a tubule of diameter 50 nm and a cylindrical actin patch of diameter 300 nm, the concentration of polymerized actin is 7 fold higher at the surface of the tubule than estimated for a homogeneous patch.