Appendices

A. Description of numerical diffusion-reaction models

A numerical reaction-diffusion model of filopodia was created for the purposes of calculating diffusion coefficients from FRAP image sequences (Fig. 5) and predicting theoretical bound fascin concentrations along growing filopodia (Fig. 8). The model consisted of a 1D tube of varying cross-section, discretized into relatively small (constant, Δx = 0.05 μm long), uniform-concentration segments along its length. Over small time steps ($\Delta t \approx$ 0.0025 s), deterministic calculations of diffusion between adjacent segments (using Fick's law) and first- and second-order kinetic reactions within a segment were made. The filopodial base was assumed held at a constant soluble fascin concentration, while the tip was a reflecting boundary (i.e. a "wall"). The simulation was thus a discretization of the 1D diffusion-reaction equations, as follows:

$$\frac{\Delta[F]_i}{\Delta t} = -\frac{2D\Delta t}{\Delta x^2}\left[\left([F]_i - \frac{[F]_{i-1}Ac_{i-1} + [F]_i Ac_i}{Ac_{i-1} + Ac_i}\right) - \left(\frac{[F]_i Ac_i + [F]_{i+1}Ac_{i+1}}{Ac_i + Ac_{i+1}} - [F]_i\right)\right] \ldots$$

$$\ldots - k_{on}[F]_i[S]_i + k_{off}[FS]_i \qquad [F] \text{ (fascin) diffusion and kinetics}$$

$$\frac{\Delta[S]_i}{\Delta t} = -k_{on}[F]_i[S]_i + k_{off}[FS]_i \qquad [S] \text{ (empty fascin binding sites on actin) kinetics}$$

$$\frac{\Delta[FS]_i}{\Delta t} = k_{on}[F]_i[S]_i - k_{off}[FS]_i \qquad [FS] \text{ (fascin-actin site complex) kinetics,}$$

where square brackets denote concentrations, the subscript i denotes the axial segment (position), Ac_i denotes the filopodial cross-sectional area of segment i, and k_{off} and k_{on} are the kinetic rate constants (given in Table 1). The following boundary conditions were applied:

$$[F]_{i=0} = [F]_{base} \qquad \text{fascin concentration is fixed at base}$$

$$\frac{\Delta[F]_{i=tip}}{\Delta x} = 0 \qquad \text{fascin gradient is zero (no diffusion) at tip}$$

$$\text{number of elements} = \frac{V}{\Delta x}t \qquad \text{filopodial length grows at rate } V \text{ in time } t$$

In the experimental determination of diffusion coefficients (Fig. 5), sequential images of soluble markers undergoing FRAP provided data on the cross-sectional areas and marker concentrations along filopodia. The total number of labeled molecules assigned to each model segment was taken as the total intensity of the pixels in the corresponding image area. Because soluble markers in the pre-bleach image were at a uniform concentration, their total segmental intensity indicated the (significantly varying) cross-sectional area Ac_i of each axial segment i. Similarly, the first post-bleach image indicated the total number of labeled molecules per segment, but in the "initial condition" state. The model was therefore initialized with the filopodial length, respective segment cross sectional areas, and initial concentrations $[F]_{i,\,(t\,=\,0)}$ equal to the total number of molecules in the first post-bleach image divided by the cross-sectional area at each segment. Filopodial length remained constant (i.e. V = 0) in both the images and these simulations. Time-dependent diffusion calculations were then carried out (no kinetics were required here), and a recovery profile of the total filopodial fluorescence with time was plotted. This profile was compared to the experimental profile measured in the same manner, and the diffusion coefficient of the model was adjusted iteratively for a profile match. The diffusion coefficient used to attain a matching profile was then reported.

Calculation of the theoretical bound fascin concentration along the length of a growing filopodium (Fig. 8) used the same technique but calculated kinetics and simulated filopodial growth. From an initial length near zero, segments were added to the tip to match the desired filopodial elongation rate V, and appeared with a constant available fascin binding site concentration $[S]_i$ and constant Ac_i, but zero occupied sites $[FS]_i$. Each additional

segment was accompanied by a shift in soluble concentration values toward the new segment, with the filopodial base segment drawing in fascin at the fixed base concentration. From an initial length near zero, each time step consisted of both diffusion calculations between segments and kinetic on- and off-reactions within them. The result was a bound fascin concentration value as a function of time and axial position.

Values measured from B16 cells were used in modeling. The soluble fascin concentration at equilibrium was equal to that of the cellular pool, $[F]_{base}$, and measured by quantitative western blotting to be 100 nM (Appendix B). To determine the total binding site concentration, we first calculated the available binding site concentration [*S*] at equilibrium. Taking advantage of the data from Fig. 7c, indicating the fraction of fascin in filopodia which are bound f_{bound}:

$$k_{on}[S][F] = k_{off}[FS]$$

$$[S] = \frac{k_{off}}{k_{on}}\frac{[FS]}{[F]} = \frac{k_{off}}{k_{on}}\frac{[FS]/([FS]+[S])}{[F]/([FS]+[S])} = \frac{k_{off}}{k_{on}}\frac{f_{bound}}{1-f_{bound}} = \frac{0.12}{0.8}\left(\frac{0.98}{0.02}\right) = 7\ \mu\text{M}$$

The same calculation using a value of f_{bound} = 0.95 results in [*S*] = 3 μM. Using this range of 3 to 7 μM, we can calculate the total binding site concentration as:

$$[S]+[FS] = \text{total binding site conc.} = [S]\left(1+\frac{k_{on}}{k_{off}}[F]\right)$$

$$= (3 \text{ to } 7\ \mu\text{M})\left(1+\frac{0.8}{0.12}0.1\right) = 5 \text{ to } 12\ \mu\text{M}.$$

We therefore initialized each added filopodial segment with [*FS*] = 0 (no bound fascin) and [*S*] = 10 μM.

B. Effective diffusion of fascin in filopodia

While we have already found that the *free* diffusion coefficients of WT fascin (i.e., in its unbound state) is 6 $\mu m^2 s^{-1}$, its *effective* diffusion coefficient describes its overall motility. That is, instead of a full description of the kinetic and diffusive processes fascin alternates between, we may more simply describe the apparent motion as diffusion with a lower, effective diffusion coefficient. In general, the 1D diffusion coefficient of a molecule is the average distance traveled squared, divided by twice the time elapsed: $D = x_{RMS}^2/(2t)$. Because fascin molecules in filopodia are only free 2 – 6% of the time (Fig. 7), the time required to travel a distance x_{RMS} is the inverse, or 50 – 17 times the interval required for non-binding molecules with the same actual diffusion coefficient. Thus, the apparent, or effective, diffusion coefficients of active/non-phosphorylatable forms of GFP-tagged and WT fascin are only 0.12 – 0.35 $\mu m^2 s^{-1}$.

C. Determination of the operating fascin:actin ratio in filopodia

Quantitative immunoblotting was used to determine the concentration of cellular fascin protein to be 100-300 nM in B16 cells (350-500nM in N2a cells). That value was converted to number of fascin molecules per cell by dividing the amount of fascin protein by molecular weight of fascin (55 kD) and Avogrado's number, yielding 1.0-2.5x10^5 molecules per B16 cell (3.0-4.6x10^5 per N2a cell). Using fluorescence microscopy, we determined the percent of total fascin molecules in a cell which localize to filopodia, 11% in B16 (29% in N2a). To calculate the number of fascin molecules in one filopodium, we divided by the average number of filopodia per cell (number of filopodia in B16 cells = 30, n = 88; number of filopodia in N2a cells = 60, n = 30), or

$$0.11 \times \frac{1.0-2.5x10^5 \text{ molecules}}{30 \text{ filopodia}} = 368-920 \text{ fascin / filopodium.}$$

Similar calculations yielded 1450-2200 fascin per N2a filopodium. We found that, on average, B16 filopodia were 3μm long (Vignjevic et al. 2006) and N2a filopodia were 8μm (current study). By assuming that filopodia contain 20 actin filaments (Lewis and Bridgeman, 1992; Mogilner and Rubinstein, 2005) and using the accepted incremental extension of an actin monomer of 2.7 nm, which gives 370 actin monomers per μm of filament, we found that the filopodia contain:

$$\frac{370\ \text{actin monomers}}{\mu\text{m}} \times \frac{(20\ \text{filaments})(3\ \mu\text{m})}{\text{filopodium}} \times \frac{\text{filopodium}}{368-920\ \text{fascin}} = 25-60\ \frac{\text{actin monomers}}{\text{fascin}}.$$

Similar calculations yielded 27-41 actin per fascin in neuronal N2a filopodia.