Supporting Information

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SI Methods

Bundle Geometry. The amount of ABP that can bind to a filament is limited, fascin binding saturates at a ratio of 1 fascin to ≈4.5 actins. At saturation an actin filament with 6 fascin binding sites therefore consists of ≈13–14 actin monomers. In a system with a given total amount of actin only a limited number of bundles consisting of n_f filaments can be obtained. Assuming that all bundles have the same thickness and all binding sites are occupied, the number of filaments in one bundle, n_f is determined by the ratio between the total number of cross-linker n_{cl}^t and actin monomers n_a^t in the system

$$n_{cl}^t/n_a^t = R = 1/u(3 \pm 3.5n_f^{-1/2}).$$

The u in this equation refers to the number of actin monomers necessary to build one hexagonal unit, the ± sign refers to whether surface occupation is included or not. For building less but thicker bundles that are fully saturated with cross-linking molecules, decreasing amounts of cross-linkers are needed. To compare the R values at which fully saturated bundles of n_f filaments are obtained with the experimentally observed values, the $D(R^*)$ curve (Fig. 1*C*) has to be translated into a $R^*(n_f)$. To obtain $R^*(n_f)$ it is assumed that $D \approx {n_f}^{1/2}$ and that the maximum bundle thickness observed in bulk networks is ≈20 filaments as observed in confinement (Fig. 2*E*). Our experimental results on the bundle thickness as a function of R^* lie below the theoretical curves (Fig. S1); for bundles with $n_f < 20$ not all possible cross-linker binding sites between the filaments in the bundle are occupied. With increasing n the degree of binding site occupation increases until at $n \approx 20$ full occupation is reached (Fig. S1).

The helical symmetry of the F-actin is described by the ratio $M = p/s$, where p is the number of monomers per crystallographic repeat and s is the number of helical turns per crystallographic repeat. The normal −13/6 symmetry is not optimal for packing on a hexagonal lattice. In the optimal symmetry the actin binding sites would be placed every 60°. In filaments with a −13/6 symmetry the difference between the optimal and actual mean position becomes as large as 13.8° (Fig. S2). In the −28/13 symmetry the maximal difference between the optimal and the actual mean position is 10.7° and the total difference between the optimal and actual position is somewhat smaller than in the −13/6 symmetry (Fig. S2). The flexibility of the fascin is apparently large enough to overcome the 10.7° required for hexagonal packing. Although the change filament twist that accompanies the binding site occupation improves the filament geometry for hexagonal packing a little bit, even the final −28/13 symmetry is not optimal for filament packing. For optimal packing on a hexagonal lattice the filaments in the bundle would have to twist even more, this is however impossible as all ABP binding sites are occupied. The suboptimal symmetry of the filaments results in build-up of stress in the bundle and is probably responsible for the limited bundle thickness.

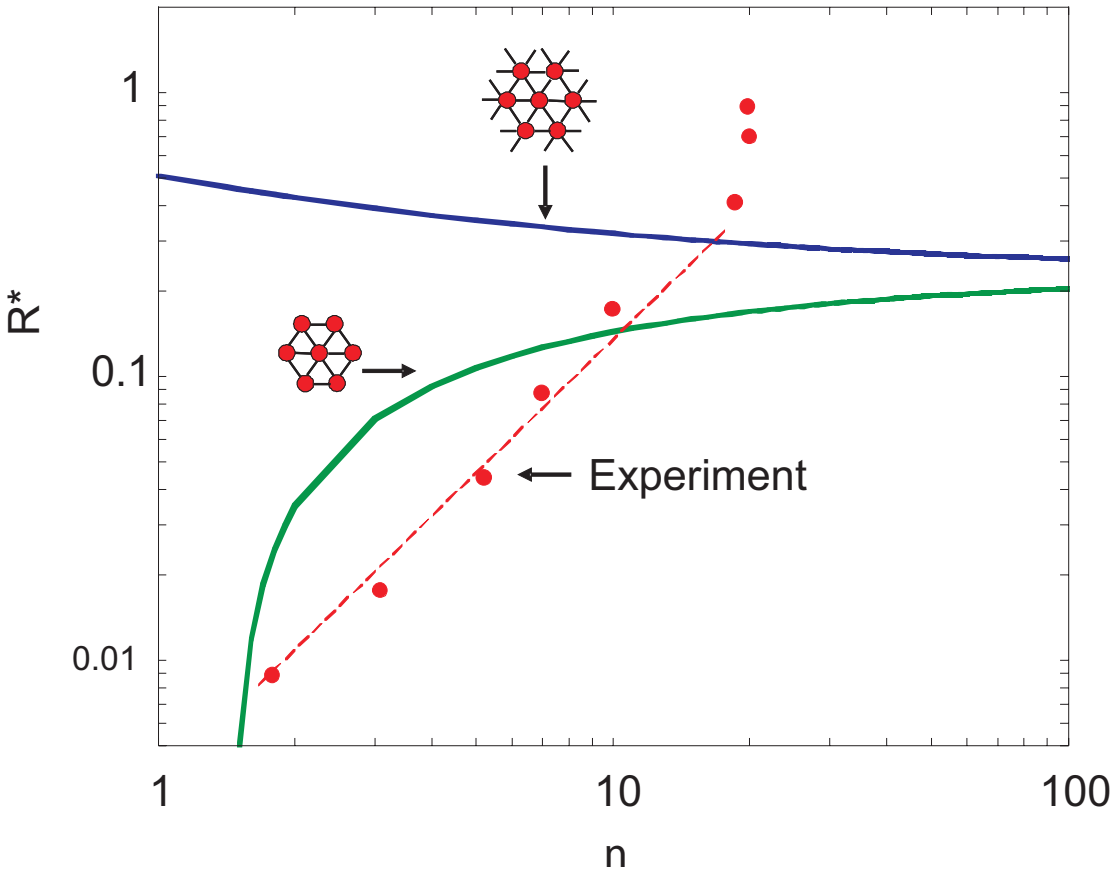

Fig. S1. Effective cross-linker over bound actin monomer ratio R^* as a function of the bundle thickness in the number of filaments n. At a constant actin concentration, a system of fully saturated bundles would require decreasing amount of cross-linkers with increasing n (blue line). Inside occupation would require increasing amounts of cross-linker (green line). In the experimentally observed bundles, the cross-linker binding sites are not fully occupied for $n < 20$ filaments (filled circles). For simplicity, u was held constant and was assumed to be 13.

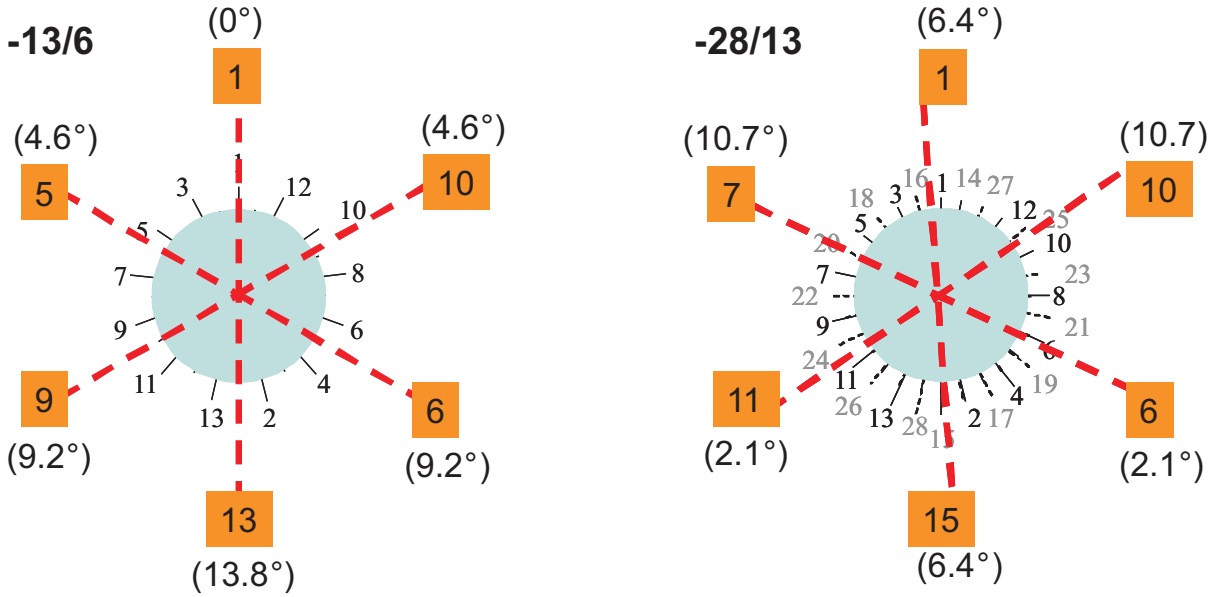

Fig. S2. Projection of actin filament in −13/6 (*Left*) or −28/13 (*Right*) symmetry. The red lines represent the ideal positions for hexagonal packing. The numbers in the orange boxes give the monomer number closest to this ideal position. The numbers between brackets indicate the difference in angle between the ideal and actual position. For the −28/13 symmetry deviations from the ideal lattice are smaller than for the −13/6 symmetry.