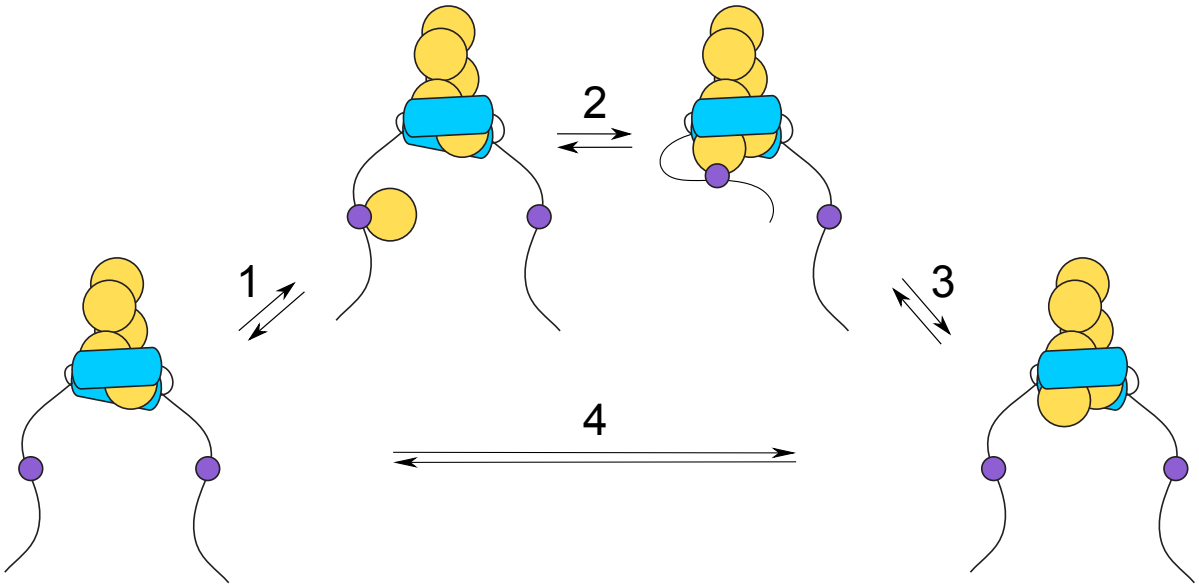
Supplemental Material



Supplemental Figure S1. Kinetic schemes for $PRF_{22}FH2$-mediated actin subunit addition. An actin monomer can be added to the barbed end of a filament bound by $PRF_{22}FH2$ via an initial interaction with FH1-tethered profilin (reaction 1), followed by FH1-mediated delivery (reaction 2) and subsequent dissociation of profilin (reaction 3). Alternatively, actin can bind the barbed end directly (reaction 4). The kinetic parameters associated with each reaction are summarized in Supplemental Table S1.

Supplemental Table S1. Kinetic model parameter values

Reaction	Description	Rates
1	Actin monomer binding to profilin	[a]$k(A+P)_{on} = 4\ \mu M^{-1}*s^{-1}$ [b]$k(A+P)_{off} = K_d(A+P)*k(A+P)_{on}\ (s^{-1})$
2	Association of actin with barbed end (delivery)	[c]$k(Delivery)_{on} = p*r_o*n_r^{-(3/2)}$ $k(Delivery)_{off} = (k(A+P)_{on}*k(Delivery)_{on}*k(P+BE)_{off}*k(A+BE)_{off})/(k(A+BE)_{on}*k(P+BE)_{on}*k(A+P)_{off})\ (s^{-1})$ (Detailed Balance) (s^{-1})
3	Dissociation of profilin from barbed end	[d]$k(P+BE)_{on} = r_o*n_r^{-(3/2)}\ (s^{-1})$ [e]$k(P+BE)_{off} = k(P_{free}+BE)_{off}\ (s^{-1})$
4	Direct binding of actin to barbed end	[f]$k(A+BE)_{on} = p*12\ (\mu M^{-1}*s^{-1})$ [f]$k(A+BE)_{off} = p*1.4\ (s^{-1})$

General reaction parameters

Parameter	Description	Value
$K_d(A+P)$	Dissociation equilibrium for FH1-profilin*actin	[g]6 μM
p	Gating factor	[h]0.5
n_r	Distance (in residues) from profilin to the FH2 domain	22 (for PD)
r_o	Association rate for two ends of unfolded polypeptide incorporating orientational effects	[i]$10^6\ s^{-1}$

[a]This rate was optimized to fit the experimental data this study, using the rate used by Vavylonis and coworkers (20 $\mu M^{-1}s^{-1}$) as a starting point for optimization [1]. The rate used in the Vavylonis study is two-fold slower than experimental rates measured with free profilin [2, 3] to account for the effects of FH1 binding on the mobility of free profilin.

[b]Perelroizen and coworkers demonstrated that free profilin and polyproline-bound profilin bind actin monomers with the same affinity [3]. Likewise, we assume that tethering profilin to the FH1 domain does not influence its affinity for actin.

[c]This value was used in the simulations in the Vavylonis study and assumes the FH1 domain is a random coil [1].

[d]This rate is similar to the delivery rate, but assumes that profilin binding to the barbed end is independent of FH2 domain gating [1].

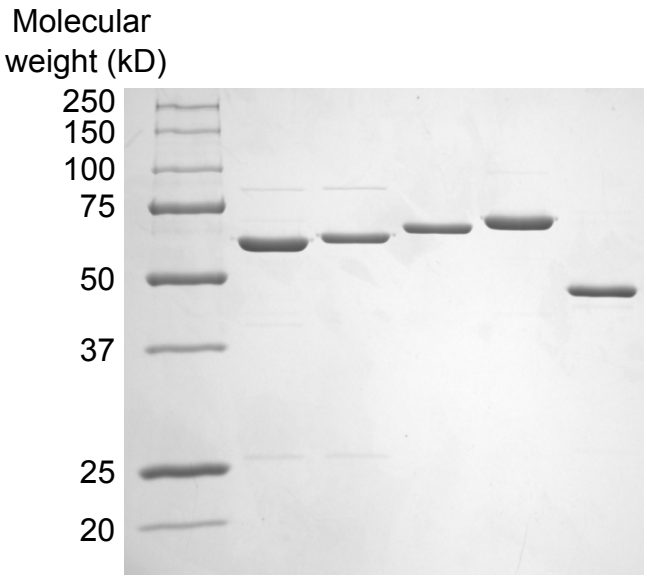
[e]This rate was used in the simulations in the Vavylonis study and was derived using the affinity of human profilin 1 for filament barbed ends (~250 μM [4, 5]). It also assumes that the FH2 domain does not influence the affinity of profilin for the barbed end [1].

[f]From Pollard [6], with the gating factor "p" as a prefactor.

[g]This value accounts for the differential binding affinities of *S. cerevisiae* profilin for unlabeled and fluorescently-labeled actin monomers (2.9 μM and 29 μM, respectively [2, 7]), enabling kinetic models to accurately reproduce experimentally-determined filament elongation rates mediated by wild-type Bni1p [1, 8, 9].

[h]This value was first reported by Kovar and coworkers [10].

[i]This parameter is a microscopic rate into which all orientational effects have been collapsed [1]. The rate of association of the two ends of an unfolded peptide is 10^7 s^{-1} [11]. Our rate is slower to account for the probability of collisions between FH1-tethered profilin and the barbed end occurring in the correct orientation to promote a binding event.



Supplemental Figure S2. SDS-PAGE analysis of purified proteins used in this study. Approximately 1 µg of each protein was loaded onto a 5%/12% Tris-HCl SDS-PAGE gel along with a ladder of protein standards (Bio-Rad Precision Plus Protein Unstained Standards). Following electrophoretic separation, proteins were visualized by staining with Coomassie Brilliant Blue. Lane 1: Ladder, Lane 2: Bni1p $PRF_{22}FH2$ (66.1 kD), Lane 3: Bni1p $PRF_{38}FH2$ (68.1 kD), Lane 4: Bni1p $PRF_{56}FH2$ (71.5 kD), Lane 5: Bni1p $PRF_{97}FH2$ (73.7 kD), Lane 6: Bni1p FH2 (49.4 kD).

Supplemental References

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