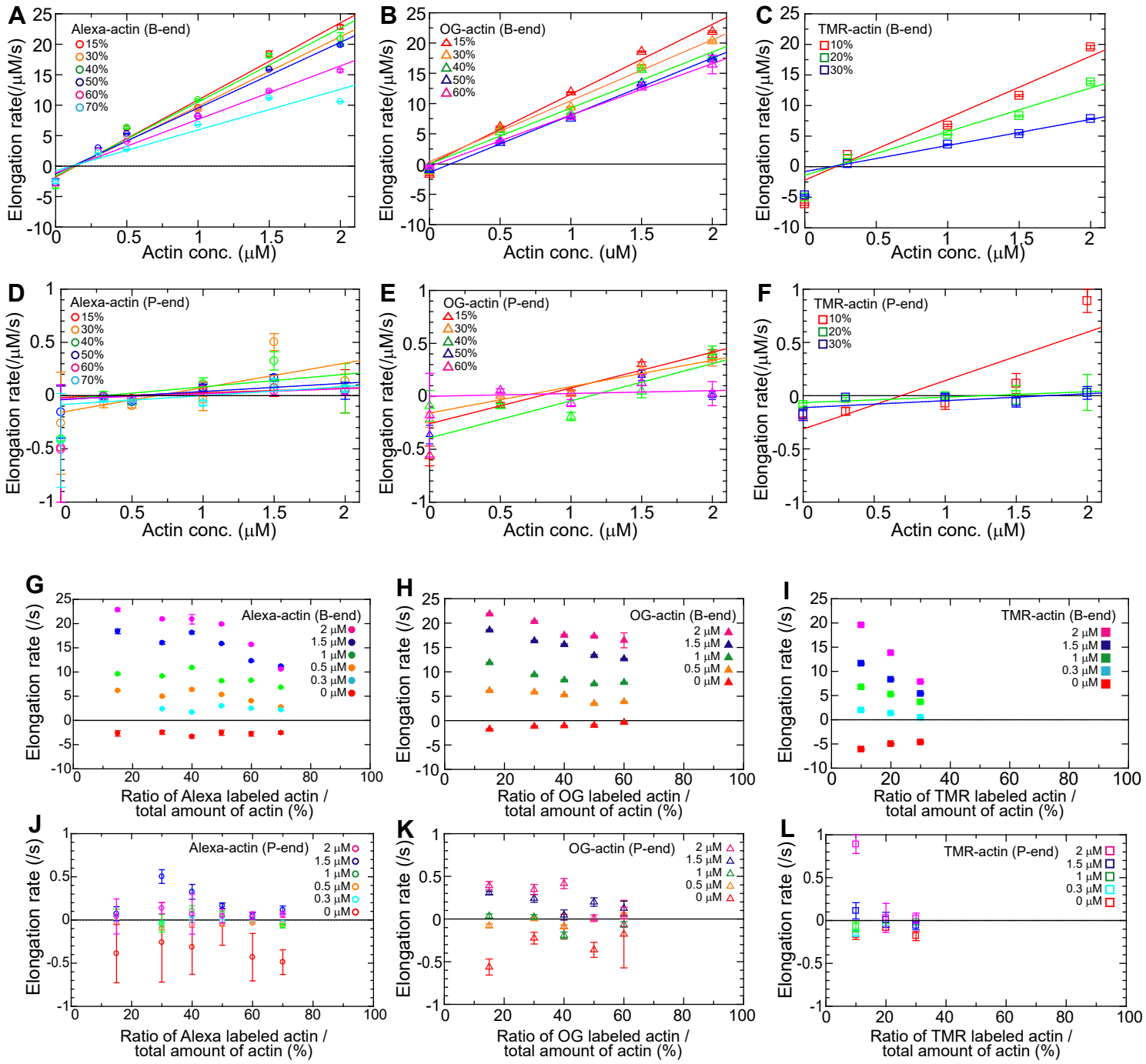
A
Alexa-actin (B-end)
15%
30%
40%
50%
60%
70%
Elongation rate(/μM/s)
Actin conc. (μM)
B
OG-actin (B-end)
15%
30%
40%
50%
60%
Elongation rate(/μM/s)
Actin conc. (uM)
C
TMR-actin (B-end)
10%
20%
30%
Elongation rate(/μM/s)
Actin conc. (μM)
D
Alexa-actin (P-end)
15%
30%
40%
50%
60%
70%
Elongation rate(/μM/s)
Actin conc. (μM)
E
OG-actin (P-end)
15%
30%
40%
50%
60%
Elongation rate(/μM/s)
Actin conc. (μM)
F
TMR-actin (P-end)
10%
20%
30%
Elongation rate(/μM/s)
Actin conc. (μM)
G
Alexa-actin (B-end)
2 μM
1.5 μM
1 μM
0.5 μM
0.3 μM
0 μM
Elongation rate (/s)
Ratio of Alexa labeled actin / total amount of actin (%)
H
OG-actin (B-end)
2 μM
1.5 μM
1 μM
0.5 μM
0 μM
Elongation rate (/s)
Ratio of OG labeled actin / total amount of actin (%)
I
TMR-actin (B-end)
2 μM
1.5 μM
1 μM
0.3 μM
0 μM
Elongation rate (/s)
Ratio of TMR labeled actin / total amount of actin (%)
J
Alexa-actin (P-end)
2 μM
1.5 μM
1 μM
0.5 μM
0.3 μM
0 μM
Elongation rate (/s)
Ratio of Alexa labeled actin / total amount of actin (%)
K
OG-actin (P-end)
2 μM
1.5 μM
1 μM
0.5 μM
0 μM
Elongation rate (/s)
Ratio of OG labeled actin / total amount of actin (%)
L
TMR-actin (P-end)
2 μM
1.5 μM
1 μM
0.3 μM
0 μM
Elongation rate (/s)
Ratio of TMR labeled actin / total amount of actin (%)

**Figure S1. Effects of fluorescent dyes on the elongation and depolymerization of filaments of ATP-actin, related to Figure 2.** Actin was labeled on cysteine 374 with Oregon green (OG) or tetramethyl rhodamine (TMR) or on lysine with Alexa488. Elongation and depolymerization of actin filaments were observed by TIRF microscopy. Depolymerization rates at 0 µM actin were measured after washing free actin monomers out of the chamber with polymerization buffer containing ATP. Rates of elongation or shortening were determined by linear fits to plots of length vs. time for individual filaments and plotted as means and ± 1 standard deviation. (A-F) Dependence of actin filament elongation rates on the concentration of ATP-actin with a range of molar ratios of labeled to unlabeled actin. Lines are fit to the positive elongation rates to estimate the association rate constants from the slopes, the critical concentrations from the X-intercept and the dissociation rate constants from the Y-intercepts. Initial depolymerization rates (first 90 s) after washing out actin monomers are plotted on the Y-axis of A-F and not used to fit lines to the positive elongation rates. (A-C) Barbed ends. (D-F) Pointed ends. (A, D) Alexa488-actin; (B, E) OG-actin; and (C, F) TMR-actin. Colors indicate the ratio of labeled actin in the inset of each panel. (G-L) Dependence of elongation rates on the fraction of actin monomers labeled with the three dyes. Positive and negative rates were measured as in A-F. (G-I) Barbed ends. (J-L) Pointed ends. (G, J) Alexa-488-actin (H, K) OG-actin (I, L) TMR-actin. Colors indicate the actin concentrations in the inset of each panel.

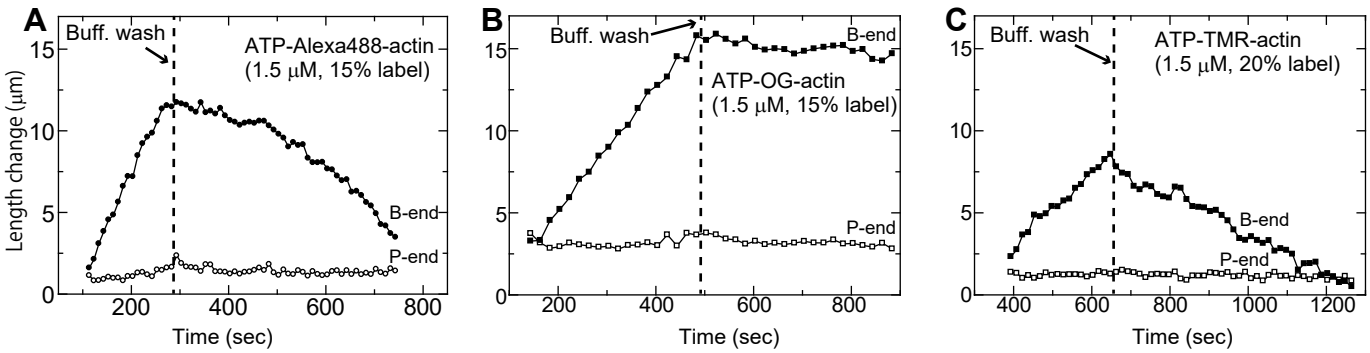
A
Buff. wash
ATP-Alexa488-actin
(1.5 μM, 15% label)
B-end
P-end
Length change (μm)
Time (sec)
B
Buff. wash
B-end
ATP-OG-actin
(1.5 μM, 15% label)
P-end
Time (sec)
C
Buff. wash
ATP-TMR-actin
(1.5 μM, 20% label)
B-end
P-end
Time (sec)

**Figure S2. Time course of polymerization and depolymerization at both ends with Mg-ATP-actin labeled with three different dyes, related to Figure 2.** TIRF microscopy was used to measure the time courses of length changes of single representative filaments during the polymerization of 1.5 μM ATP-actin, followed by washing out actin monomers with polymerization buffer containing 1.5 μM ATP at the times indicated by the vertical dashed lines to induce depolymerization. Length changes were measured from the fiducial point to each end of these filaments labeled with 3 different dyes. (A) 15% Alexa488-actin measured every 10 s; (B) 15% OG-actin measured every 20 s; and (C) 15% TMR-actin measured every 15 s. Filled symbols for barbed ends and open symbols for pointed ends.

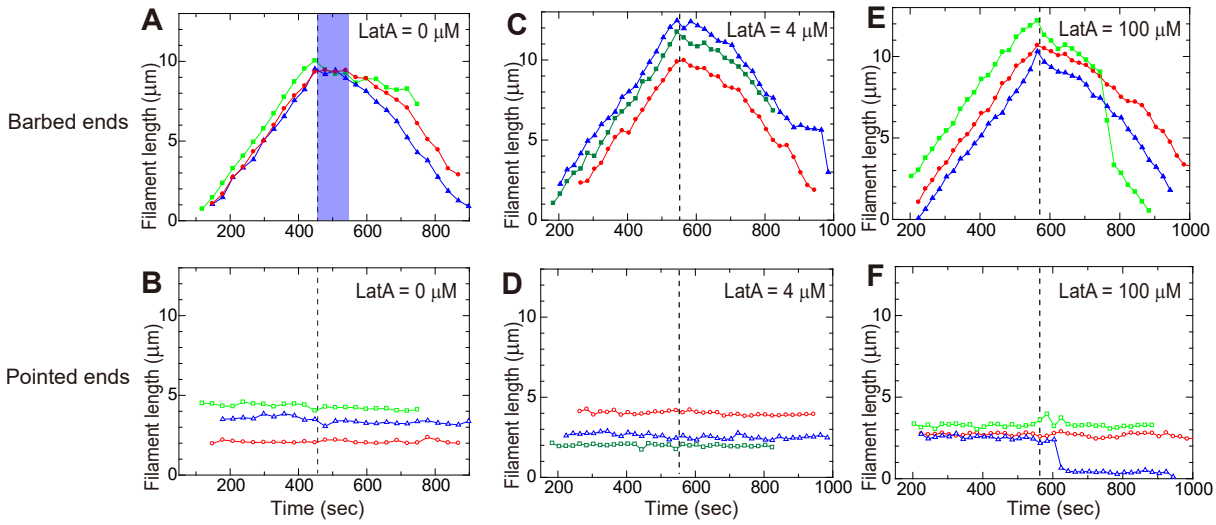
Barbed ends
Pointed ends
A
B
C
D
E
F
LatA = 0 μM
LatA = 4 μM
LatA = 100 μM
Filament length (μm)
Time (sec)
0
5
10
200
400
600
800
1000

**Figure S3. LatA promotes depolymerization of Alexa488-ATP-actin filaments, related to Figure 2.** Same experiments as Figure 2 were performed using 30% Mg-ATP-Alexa488 labeled actin at 1 μM total actin. Plots show time courses of length changes at the ends of 3 typical actin filaments. Free actin monomers were washed out at the times indicated by the vertical dashed lines and replaced by ATP-containing polymerization buffer with a range of LatA concentrations. **(A, C, E)** Barbed ends. The blue bar in (A) indicates the 90 s used to measure the initial slow depolymerization phase. **(B, D, F)** Pointed ends. **(A and B)** no LatA, **(C and D)** 4 μM LatA and **(E and F)** 100 μM LatA**.** Length changes from barbed- (closed symbols) and pointed ends (open symbols) of individual actin filaments were shown with matched colors.