Supplemental Information

Molecular Origins of Cofilin-linked Changes in Actin Filament Mechanics

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Structure Refinement

The structure refinement procedure contains two steps. The first step involves backbone refinement using the torsional optimization protocol developed by Haddadian et al..[1] This protocol converts the backbone dihedral angles into statistically more probable angles in preferred regions of Ramachandran map that are specific to each amino acid type and its neighbors. The procedure employs five energy functions: 1) a neighbor-dependent torsional statistical potential (TSP); 2) a metric for the similarity to the input reference structure; 3) an H-bond potential; 4) the repulsive portion of the Cbeta-level statistical potential designed to prevent steric clashes; and 5) a neighbor-independent TSP. A webserver has been constructed for this task (http://godzilla.uchicago.edu/). The actin subunit, chain A of PDB bank entry 3J0S and the cofilin subunit, chain N of 3J0S, have been processed by the server for backbone refinement. The RMSD of the refined actin-cofilin pair to chain A-N of the starting structure is 0.8Å. Then, using this backbone optimized structure, an actin filament decorated with cofilin is constructed and fit into the experimental real-space electron density (EM-5354) using the molecular dynamics flexible fitting (MDFF[2]) module of program NAMD.

This process improves the cofilin-actin pair structure compared to the deposited model (PDB entry 3J0S). Ramachandran outliners decrease from 3.54% to 2.09%, Ramachandran favored dihedral angles increase from 92.09% to 95.06%, the number of hydrogen bond grows from 156 to 206, and the number of poor rotamers diminishes from 7.16% to 4.59% (data summarized in SI Table 1). The cross-correlation coefficient between the target density map and the structures increases from 0.83 before to 0.85 after refinement. The actin-cofilin binding site interface in this improved model remains almost unchanged (data summarized in SI Table 2), except for the second F-actin binding site. The average atom-atom distance (not including hydrogen atoms) of this binding site increases by about 2 Å, displaying a tendency towards breakage as observed in the MD simulation.

SI Table 1 Quality of structures before and after refinement procedure.

	Cryo-EM Structure PDB entry 3J0S	Refined Structure
Ramachandran outliners[a]	3.54%	2.09%
Ramachandran favored[a]	92.09%	95.06%
Poor Rotamers[a]	7.16%	4.59%
Number of H-bond (chain A and chain N only)[b]	156	206

[a]Data from webserver molprobity.biochem.duke.edu

[b]Data from webserver godzilla.uchicago.edu

SI Table 2 The minimal inter-residue atom distance (not including hydrogen) before and after refinement procedure.

Mini-distance(Å)	Actin Residues	Cofilin Residues	Cryo-EM Structure	Refined Structure
G-actin BS-1	143-147	112-119	3.41	2.86±0.05
	343-346	112-119	3.37	3.44±0.03
G-actin BS-2	349-354	1-5	2.74	2.90±0.07
	349-354	41-46	4.06	3.90±0.22
F-actin BS-1	21-28	94-98	3.30	2.68±0.06
	90-96	94-98	3.03	2.99±0.15
	90-96	19-21	3.35	3.08±0.05
F-actin BS-2	240-244	154-158	4.85	6.71±0.09

[a]Mean values are the average of minimal inter-residue atom distance (not including hydrogen) of all actin-cofilin pair; errors are the standard deviation of the mean.

Figure S1 Evolution of crossover length for bare and cofilactin filaments, demonstrating that both simulations are have equilibrated (after ~25 ns for cofilactin) and that both filaments are stable（data for bare filament from 50-105 ns portion of simulation).

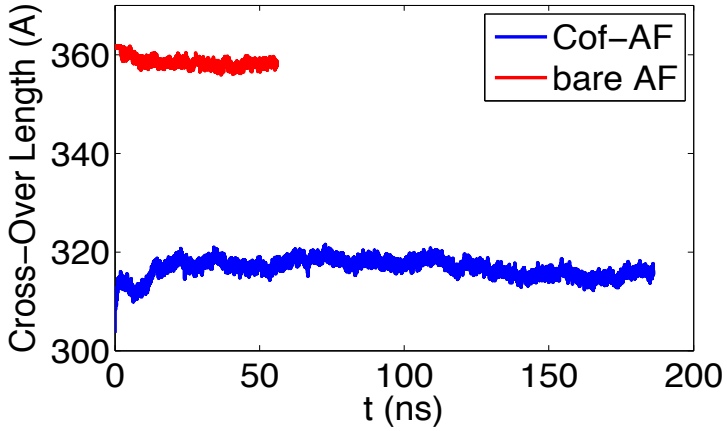
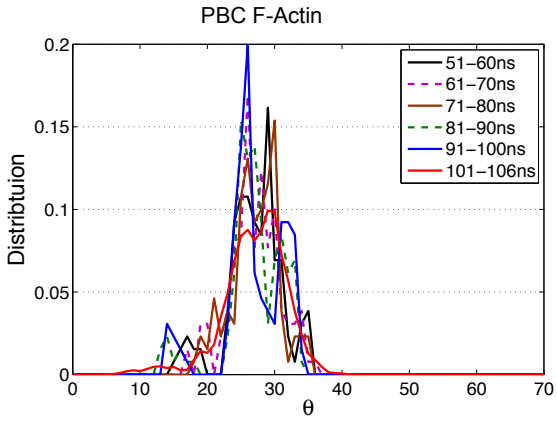


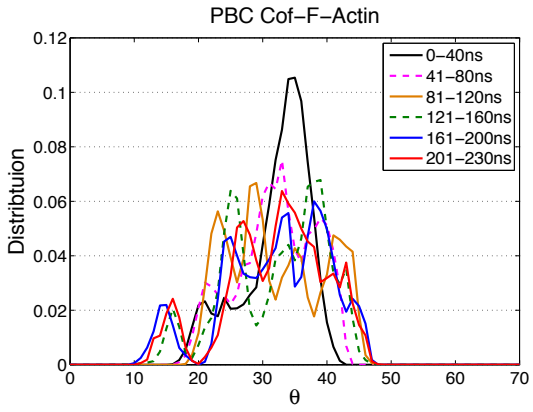
Figure S2 Time evolution of distribution of twist angle between nearest subunit pairs shows that the distribution is evolving and becomes broader with time; the distribution seems to stop expanding after 50 ns and 120 ns for bare and cofilactin filament, respectively.

(a)



Stabilized, no big difference

(b)



Stabilized after 120 ns

Figure S3 (a) RMSD vs. time of bare and cofilactin filaments, together with Figures S1 and S2, the system reaches equilibrium states after ~50 ns and ~120 ns for bare and cofilactin filaments, respectively. (b) Persistence length for each frame of last 10 ns; (c) *std* of angles between all nearest subunit pairs vs. time for cofilactin filament; (d) Time evolution (last 40 ns) of the angles between nearest subunit pairs shows each angle is trapped locally.

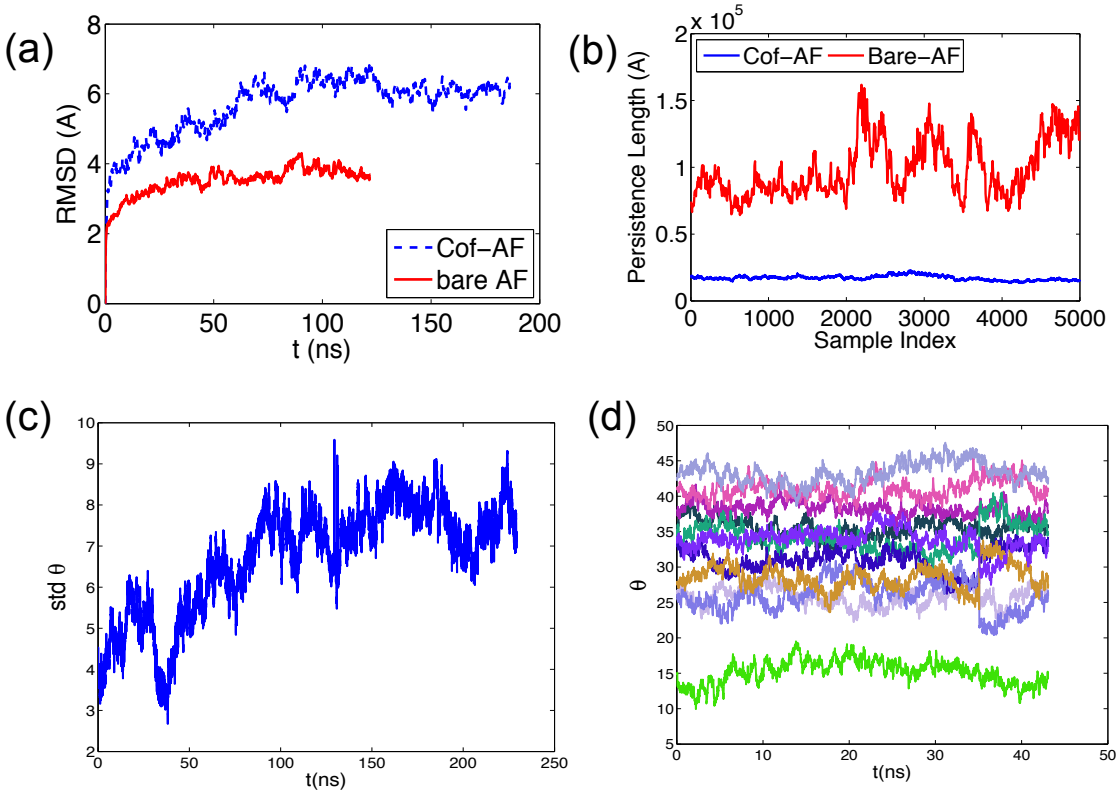


Figure S4. Binding sites 1 and 2 contribute to interactions between actin (cyan) and cofilin (green). Binding site 1, i.e. G-actin Binding site 1, consists of actin residues 143-147 in blue, 343-346 in red, and cofilin residues 112-119 in yellow; Binding site 2 , i.e. G-actin Binding site 2, consists of actin residues 349-354 in magenta, cofilin residues 1-5 in black and 41-46 in orange.

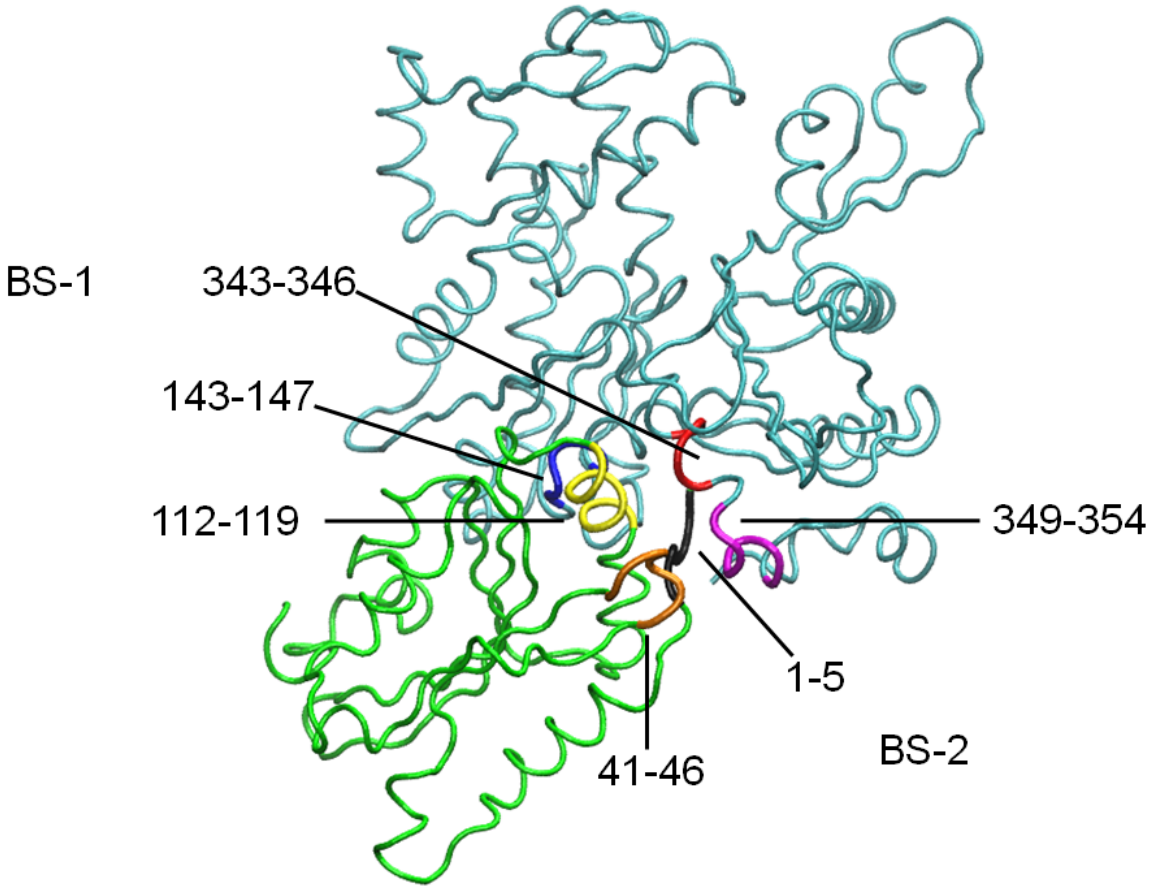


Figure S5. Binding sites 3 and 4 contribute to interactions between actin (cyan) and cofilin (green). Binding site 3, i.e., F-actin Binding site 1, consists of actin residues 21-28 in black, 90-96 in orange, cofilin residues 19-21 in red, and 94-98 in yellow; Binding site 4, i.e., F-actin Binding site 2, consists of actin residues 240-244 in magenta, and cofilin residues 154-158 in blue.

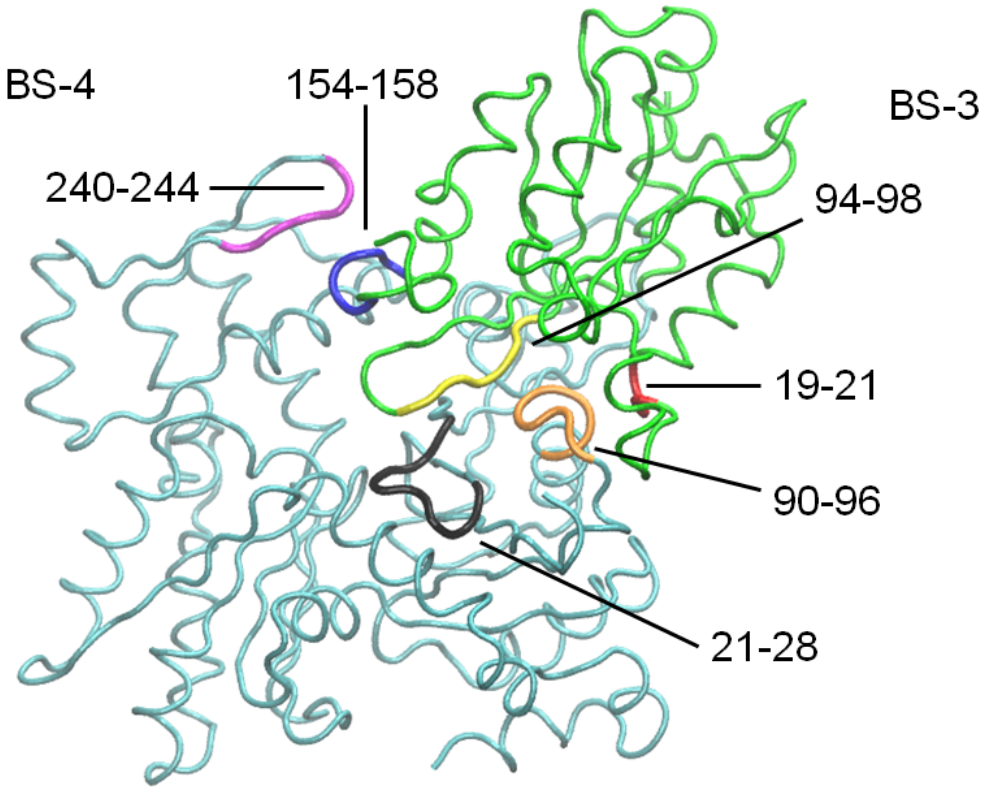
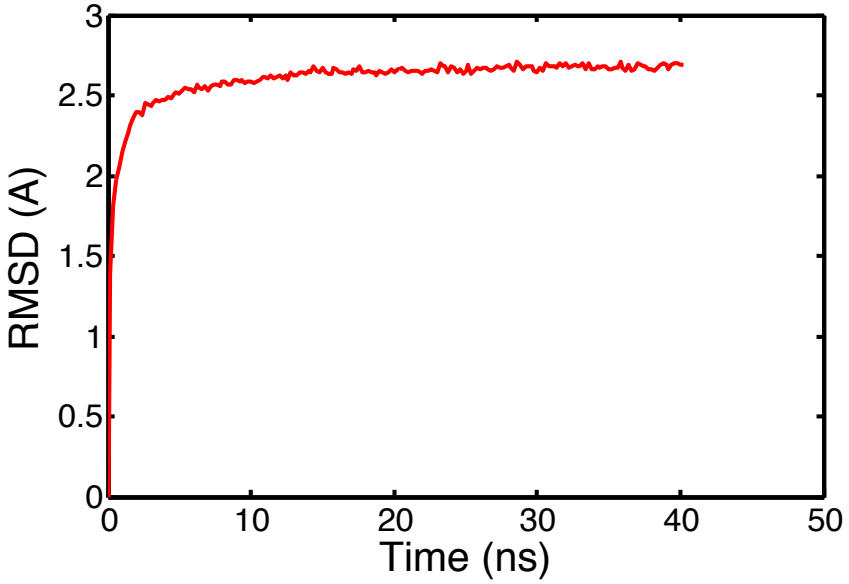


Figure S6. The RMSD evolution of cofilactin simulation at temperature 123K shows that simulation system enters the equilibrium state after ~20ns. The RMSD is converging to 2.7A, much smaller than that at 310K (~6A), shown in Figure S3(a).



References:

1. Haddadian, E. J., Gong, H. P., Jha, A. K., Yang, X. J., DeBartolo, J., Hinshaw, J. R., Rice, P. A., Sosnick, T. R. & Freed, K. F. (2011). Automated real-space refinement of protein structures using a realistic backbone move set. *Biophysical Journal* **101**, 899-909.
2. Trabuco, L. G., Villa, E., Mitra, K., Frank, J. & Schulten, K. (2008). Flexible fitting of atomic structures into electron microscopy maps using molecular dynamics. *Structure* **16**, 673-683.