Supplementary Figures to:

Autoinhibition of the formin Cappuccino in the absence of canonical autoinhibitory domains

Batbileg Bor1, Christina L. Vizcarra2, Martin L. Phillips2 and Margot E. Quinlan2,3,*

1Molecular Biology Interdepartmental Program, University of California Los Angeles, Paul D. Boyer Hall, 611 Charles E. Young Drive East, Los Angeles, California 90095-1570, USA.

2Department of Chemistry and Biochemistry, University of California Los Angeles, 607 Charles E. Young Drive, Los Angeles, California 90095, USA.

3Molecular Biology Institute at UCLA, Paul D. Boyer Hall, 611 Charles E. Young Drive East, Box 951570, Los Angeles, California 90095-1570, USA.

*Correspondence should be addressed to M.E.Q. (margot@chem.ucla.edu).

Running title: Autoinhibition of Cappuccino

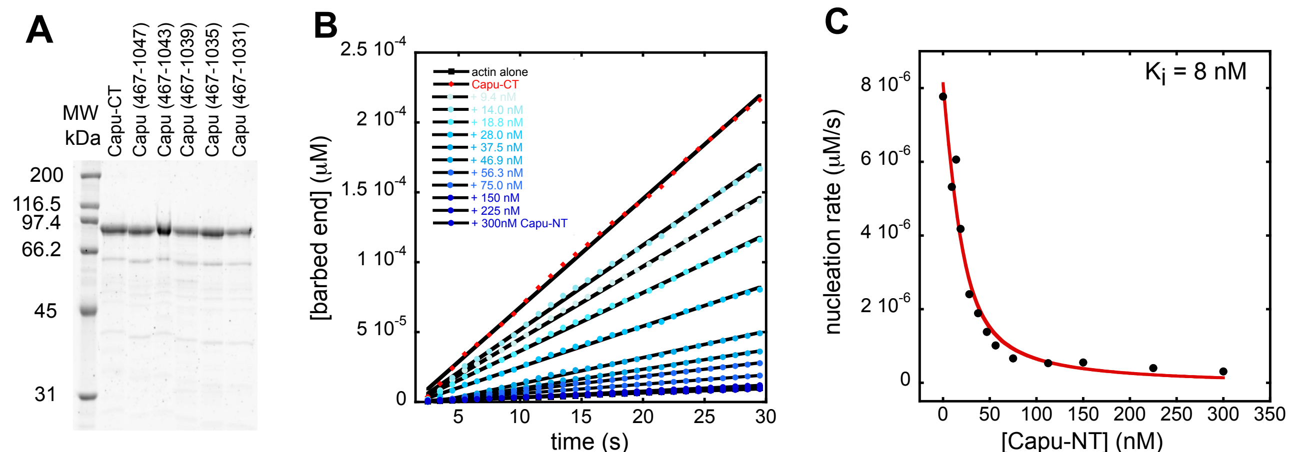


Figure S1. Capu-NT inhibits the nucleation activity of Capu-CT. (A) Purified Capu C-terminal truncations visualized on a Coomassie stained SDS-PAGE gel. (B) Analysis of Capu-CT's nucleation rate in the presence of Capu-NT. The concentration of barbed ends, calculated from the traces in Figure 1C, was plotted versus time for the initial 30 s, and the slopes of these lines were taken as the nucleation rate. (C) Nucleation rates are plotted as a function of Capu-NT concentration to determine the nucleation inhibition constant (K_i = 8 nM). This is very similar to the K_i calculated in Figure 1D.

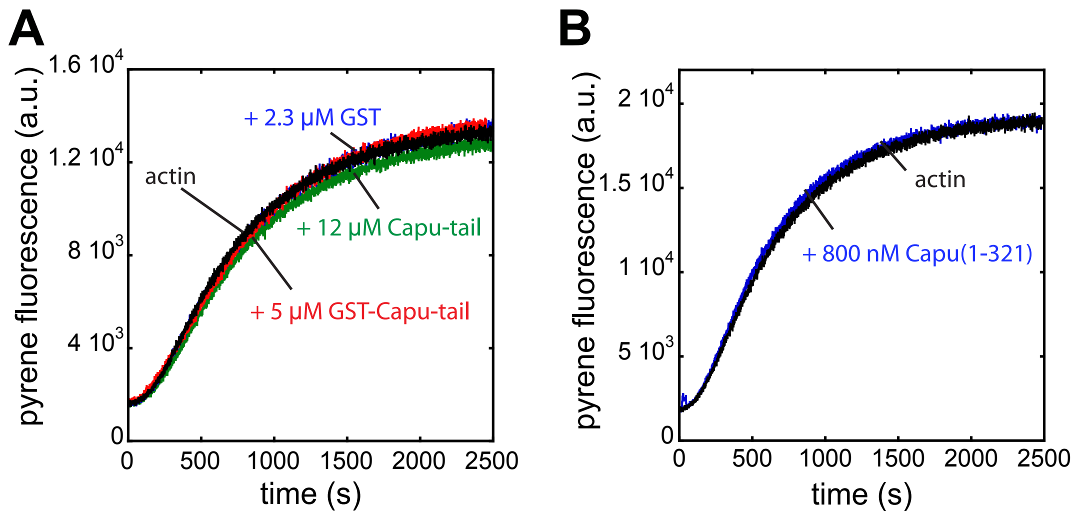
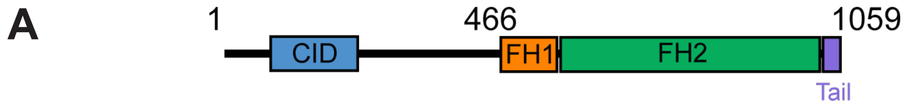


Figure S2. (A) Control experiments show that 12 μM Capu-tail (green), 2.3 μM GST (blue) or 5 μM GST-Capu-tail (red) had no effect on actin polymerization. (B) Control experiment shows that 800 nM Capu(1-321) had no effect on actin polymerization.

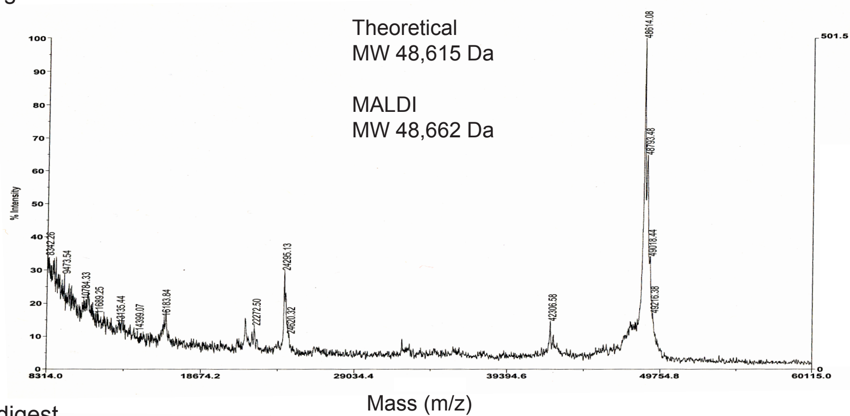
A



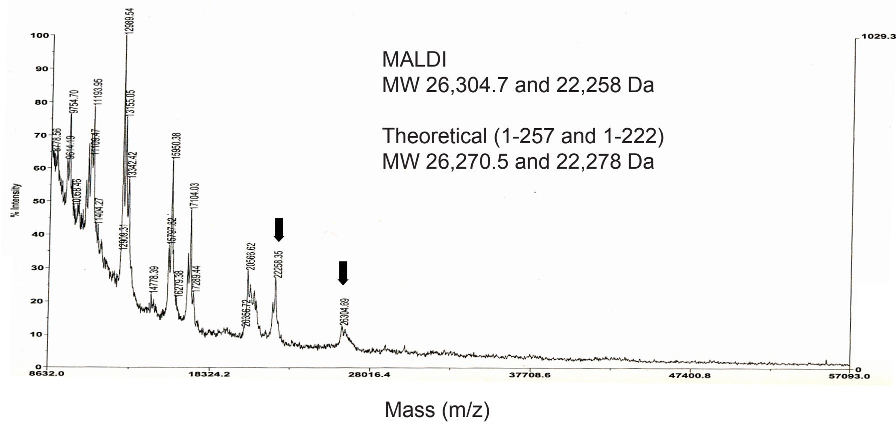
	50nM	100nM	150nM
Capu-NT	96%	---	---
50-466	76%	---	---
60-466	91%	---	---
80-466	74%	---	---
105-466	17%	52%	---
271-466	---	---	35%
318-466	---	---	11%
350-466	---	---	15%
380-466	---	---	13%
1-402	25%	---	---
1-350	45%	84%	---
1-321	80%	---	---
1-105	0%	17%	21%
25-185	23%	41%	52%
25-321	40%	---	---
50-321	0%	31%	---
60-321	---	---	61%
105-321	44%	---	---
80-350	0%	32%	47%
105-350	---	---	30%
197-350	---	---	7.2%
105-380	---	---	28%
197-380	---	---	13%

B

Pre-digest



Post-digest



Mass (m/z)

Figure S3. Mapping the CID domain. (A) Summary of the Capu-NT truncations and their Capu-CT inhibition activities. Three concentrations (50, 100, 150 nM) of various truncated Capu-NT constructs were added to the pyrene assay containing 20 nM Capu-CT. Inhibition activities were quantified by comparing the $t_{1/2}$ where Capu-CT alone is 0% and actin alone is 100% inhibited. Conditions not tested are indicated by '---'. Truncations from the N-terminus of Capu-NT quickly lost inhibition activity, suggesting the CID is near the beginning of Capu-NT. Truncations from the C-terminus of Capu-NT had varying effects. A few constructs break apparent trends. Notably Capu(50-466), Capu(1-402) and Capu(1-350). We propose that local structures are disrupted in these constructs causing their activity to be lower than expected. For this set of data, concentrations of all Capu-NT truncations were determined using a Bradford protein assay (Biorad). Concentrations for all other experiments described were determined as described in Methods. We reasoned that the comparison within this set of data is valid, although not directly comparable to the data in Figure 3A. (B) Mass spectra before and after tryptic digest. Pre- and post-digest samples of 3.2 μM Capu-NT were analyzed by MALDI. The pre-digested sample had one clear peak corresponding to Capu-NT at 48,662 Da (calculated MW is 48,614 Da). We also see a weaker peak at 24,295 Da, which is probably doubly charged Capu-NT. From the post-digest spectra, we were interested in the highest MW bands, which were two peaks at 26,305 and 22,258 Da (shown by arrows). N-terminal sequencing showed that the higher MW bands from the digest began at residue 1. Combining this information and the expected trypsin cut sites present in Capu-NT, we predicted that the highest band was Capu(1-257) (MW = 26,270 Da) or Capu(1-222) (MW = 22,278 Da).

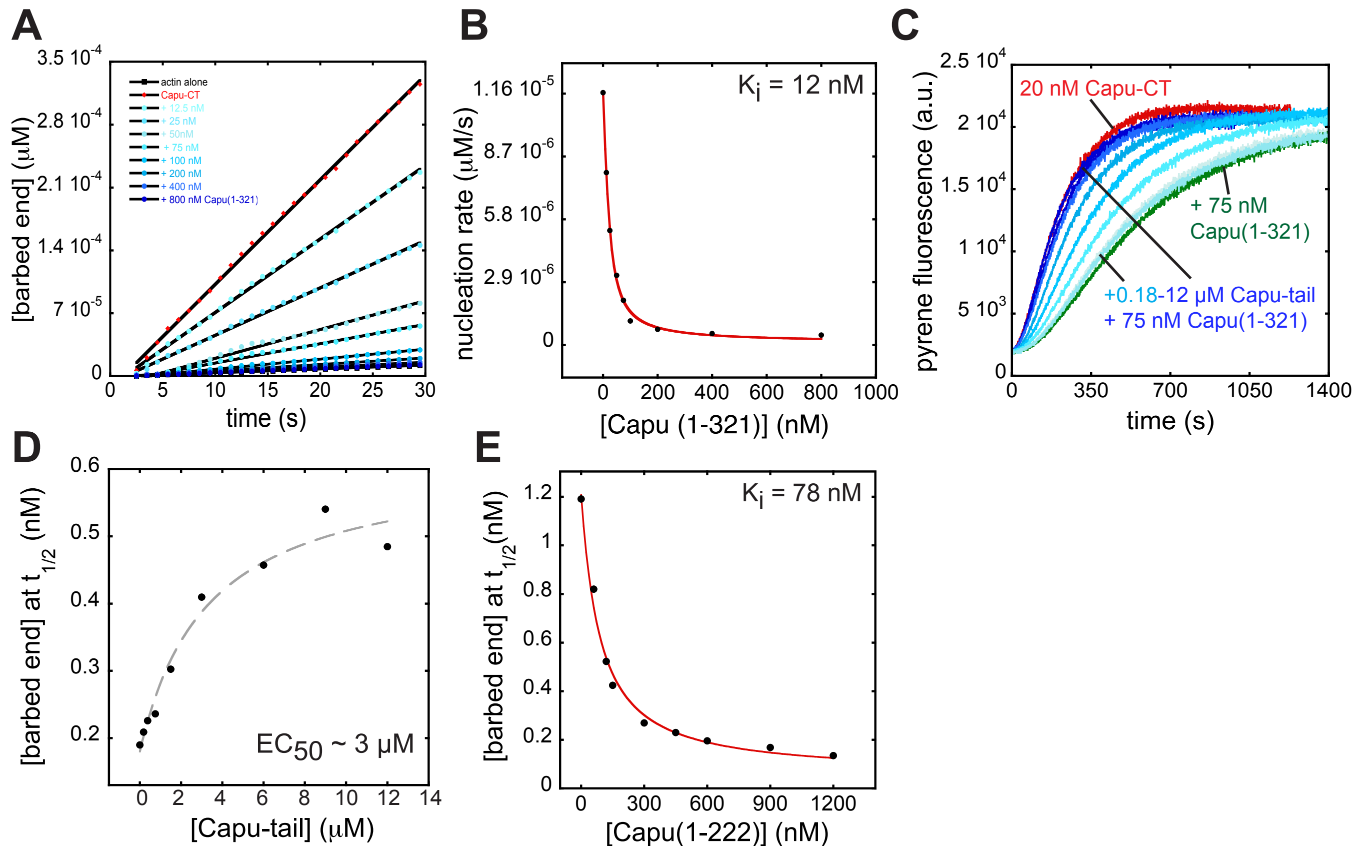


Figure S4. Analysis of the inhibitory activity of Capu(1-321) and Capu(1-222). (A-B) The data for the Capu(1-321) titration experiment in Figure 3B were fit as in Figure S1B–C. The analysis yielded a K_i of 12 nM based on nucleation rates. (C) Competition experiment with Capu(1-321), Capu-tail, and Capu-CT. Similar to Figure 2B, 0.18-12 μM Capu-tail (shown with increasing shades of blue) were added into 20 nM Capu-CT (alone in red) and 75 nM Capu(1-321) (alone in green). (D) Analysis of data from (C) was carried out as described in Figure 2C, showing EC_{50} ~3 μM. (E) The K_i of Capu(1-222) was determined as in Figure 1D, yielding a K_i of 78 nM. This is an overestimate of the affinity due to weak inhibition of actin polymerization by Capu(1-222).

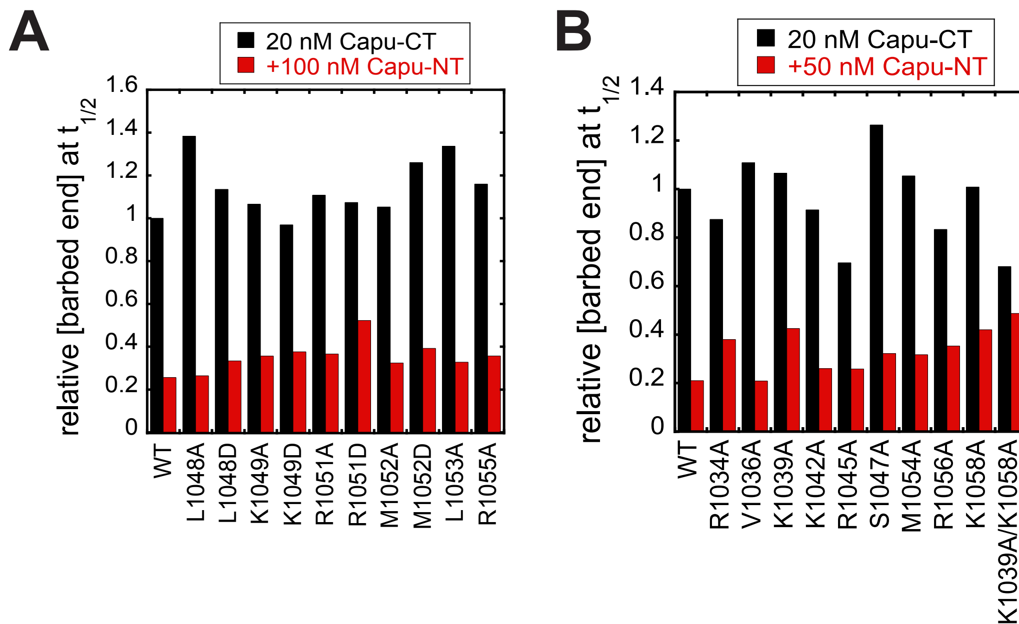


Figure S5. Mutational analysis of the Capu-CT/Capu-NT interaction. (A and B) Various mutations in the C-terminus of Capu-CT were tested for their effect on inhibition by Capu-NT. The two bar graphs show the summary of four experiments from four separate days. To compare the potency of Capu-NT inhibition from different days, wild type Capu-CT barbed end production was adjusted to an arbitrary value of 1, and the other traces were adjusted relative to the wild type data on the day they were measured. Black bars are wild type or mutant Capu-CT alone and red bars are with the indicated amount of Capu-NT.

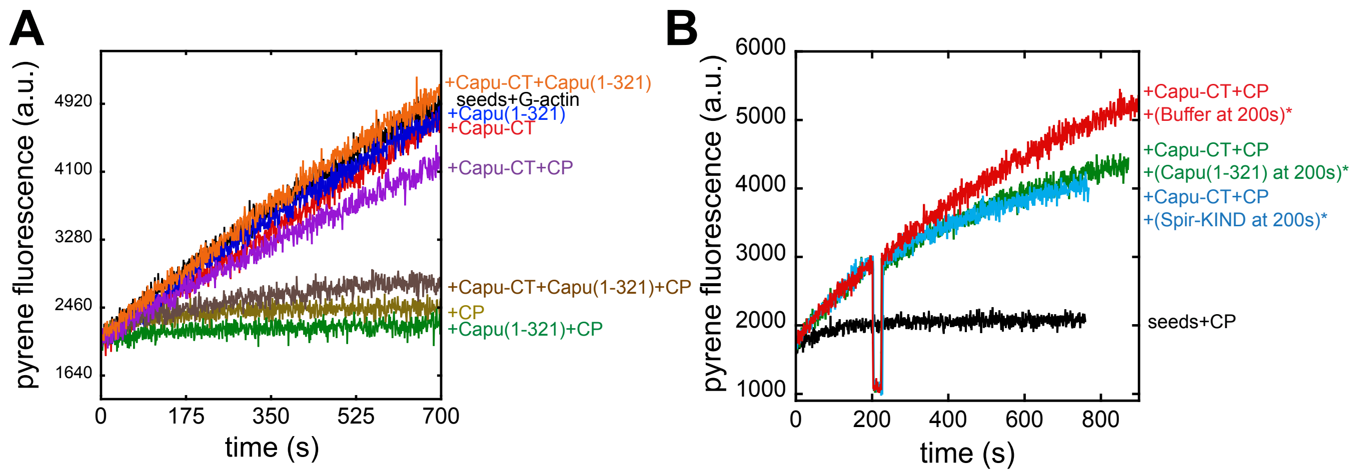


Figure S6. The effect of Capu(1-321) on processive elongation. (A) Example of pyrene fluorescence traces for each data set shown in Figure 5C. Data points between 200-700 s were fit with a line to obtain an elongation rate. (B) Essentially the same experiment as in (A) was carried out except that 2 μM Capu(1-321), 2 μM Spir-KIND or buffer were added 200 s after elongation was initiated (*). Adding a buffer control mid-assay had minimal effect on the barbed end protection by Capu-CT, but addition of either Capu(1-321) or Spir-KIND slowed elongation.

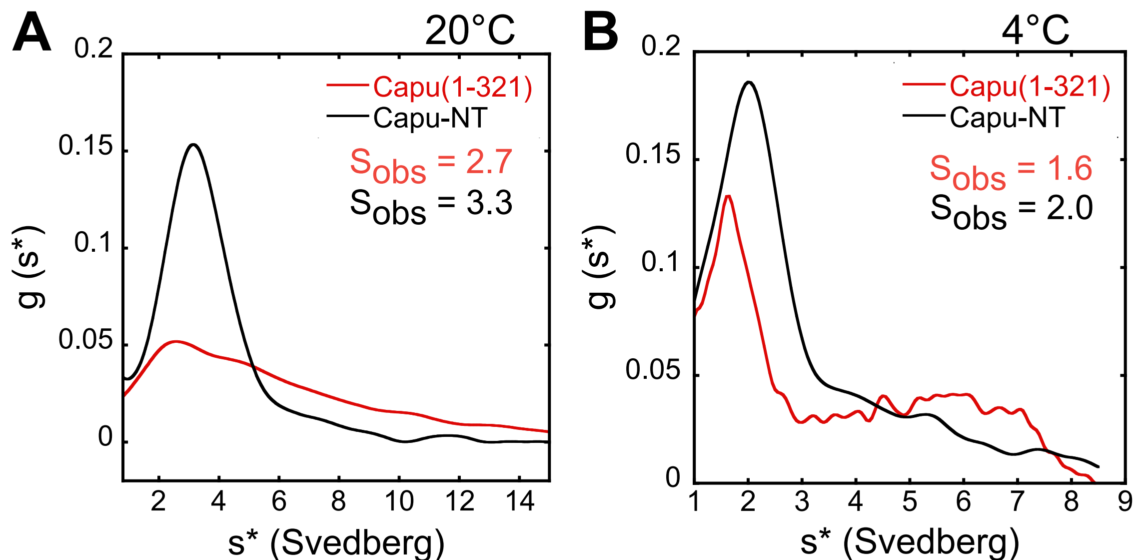
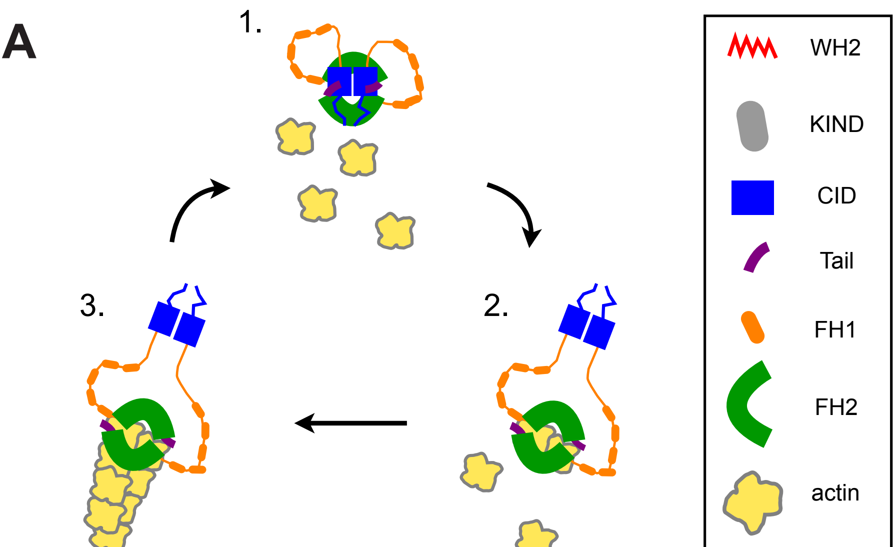


Figure S7. Capu-NT is an elongated dimer. (A) Velocity sedimentation at 20°C showed a single peak that corresponds to a sedimentation coefficient of 3.3 S for Capu-NT, indicating one dominant form of Capu-NT. The Capu(1-321) peak (2.7 S) had multiple faster sedimenting shoulders. SDS-PAGE analysis of the samples before and after centrifugation showed that Capu(1-321) degraded during the experiment (data not shown). (B) Because Capu(1-321) was not stable for the duration of the 20°C experiment, we repeated the experiment at 4°C. Both Capu-NT and Capu(1-321) had single peaks that corresponded to sedimentation coefficient of 3.3 S for Capu-NT and 2.6 S for Capu(1-321) after correction for the viscosity and density at 4°C (before correction, Capu-NT is 2.0 S and Capu(1-321) is 1.6 S), indicating one dominant form of Capu-NT or Capu(1-321). In both experiments, Capu-NT (2.4 μM) or Capu(1-321) (5 μM) were spun at 55,000 rpm for 3 hours.


A
1.
2.
3.
WH2
KIND
CID
Tail
FH1
FH2
actin

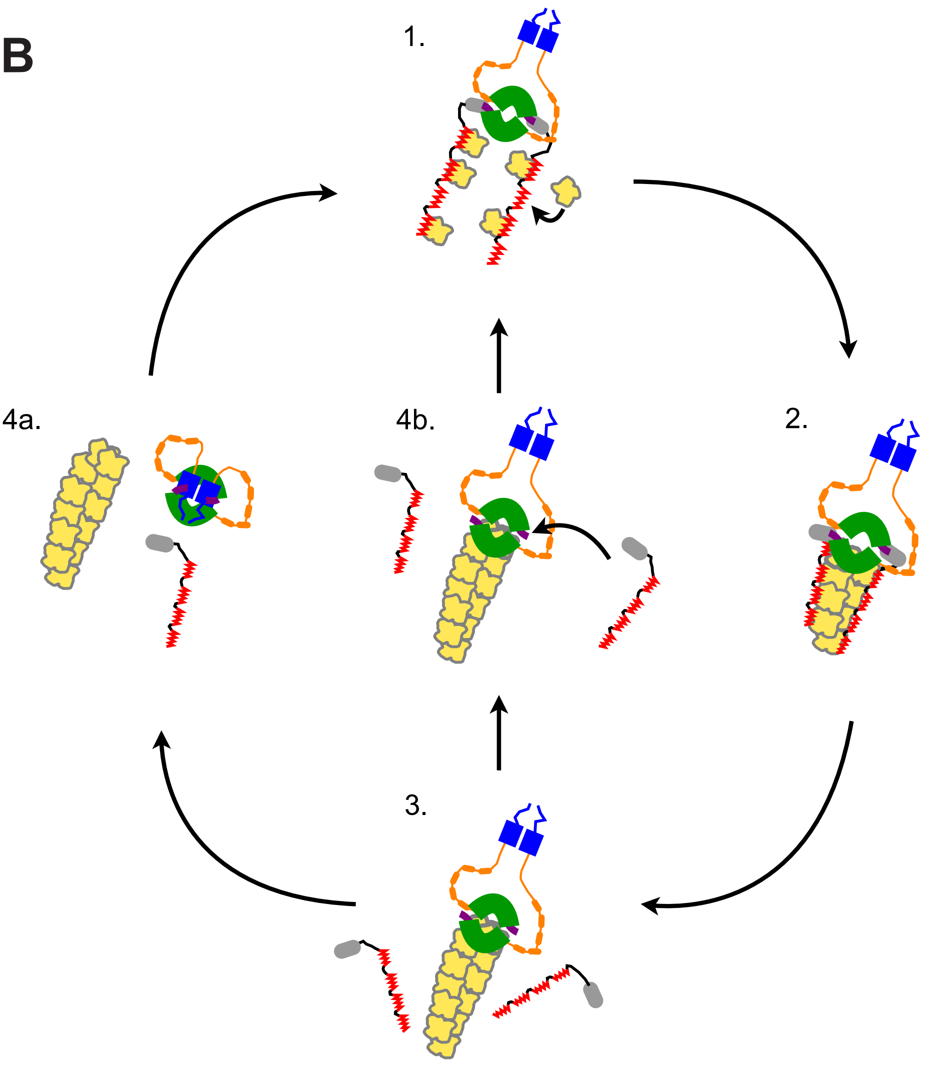

B
1.
2.
3.
4a.
4b.

Figure S8. Models of Capu regulation. (A) In the absence of Spir, Capu is regulated by autoinhibition. In state 1, Capu is in its autoinhibited confirmation and does not nucleate or associate with barbed ends of filaments. In state 2, Capu is activated by some external signal and nucleates an actin filament. Once Capu nucleates actin filaments, it stays bound to the barbed end (state 3) until it dissociates either spontaneously or in response to an external cue. Once it dissociates, it could re-form the autoinhibited conformation (state 1) unless the autoinhibitory interaction is blocked by a regulatory protein that is bound to the N-terminus or by post-translational modification. (B) In the presence of Spir, there are several potential models of how actin filament assembly is regulated. We have illustrated two here. In state 1, Capu and Spir are bound to each other and recruit actin monomers to form a filament nucleus (state 2). Based on previous biochemical data, Spir and Capu do not form an elongation complex. Therefore, in order for the nascent filament to elongate with Capu-bound to the barbed end (state 3), their interaction must be broken, either by the filament or by an external cue. Eventually, Capu may dissociate from the barbed end spontaneously or as a result of another regulator that controls filament length (state 4a). This factor could be Spir (state 4b), acting as an elongation regulator similar to the Bud14/Bnr1 interaction in yeast (Chesarone et al., 2009). If Capu-CT dissociates from the barbed end without Spir binding, autoinhibition could keep Capu in an inactive form (state 4a) until Spir binds and re-forms the nucleation complex (state 1). Whether post-translational modifications or additional regulators control this cycle is currently unknown.