Supplemental Materials

Molecular Biology of the Cell

Lacy et al.

Single-molecule imaging of the BAR domain protein Pil1preveals filament-end dynamics

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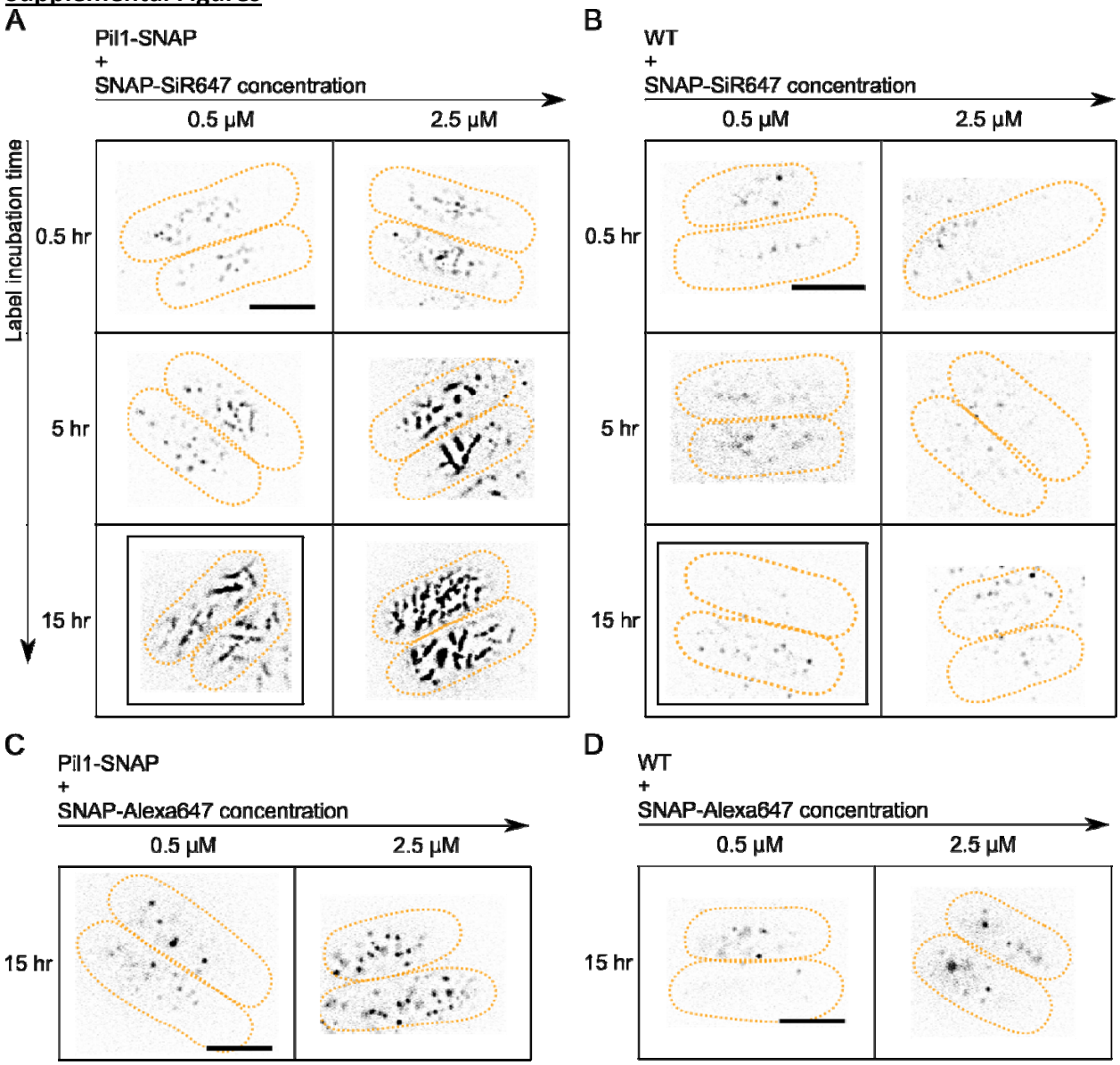
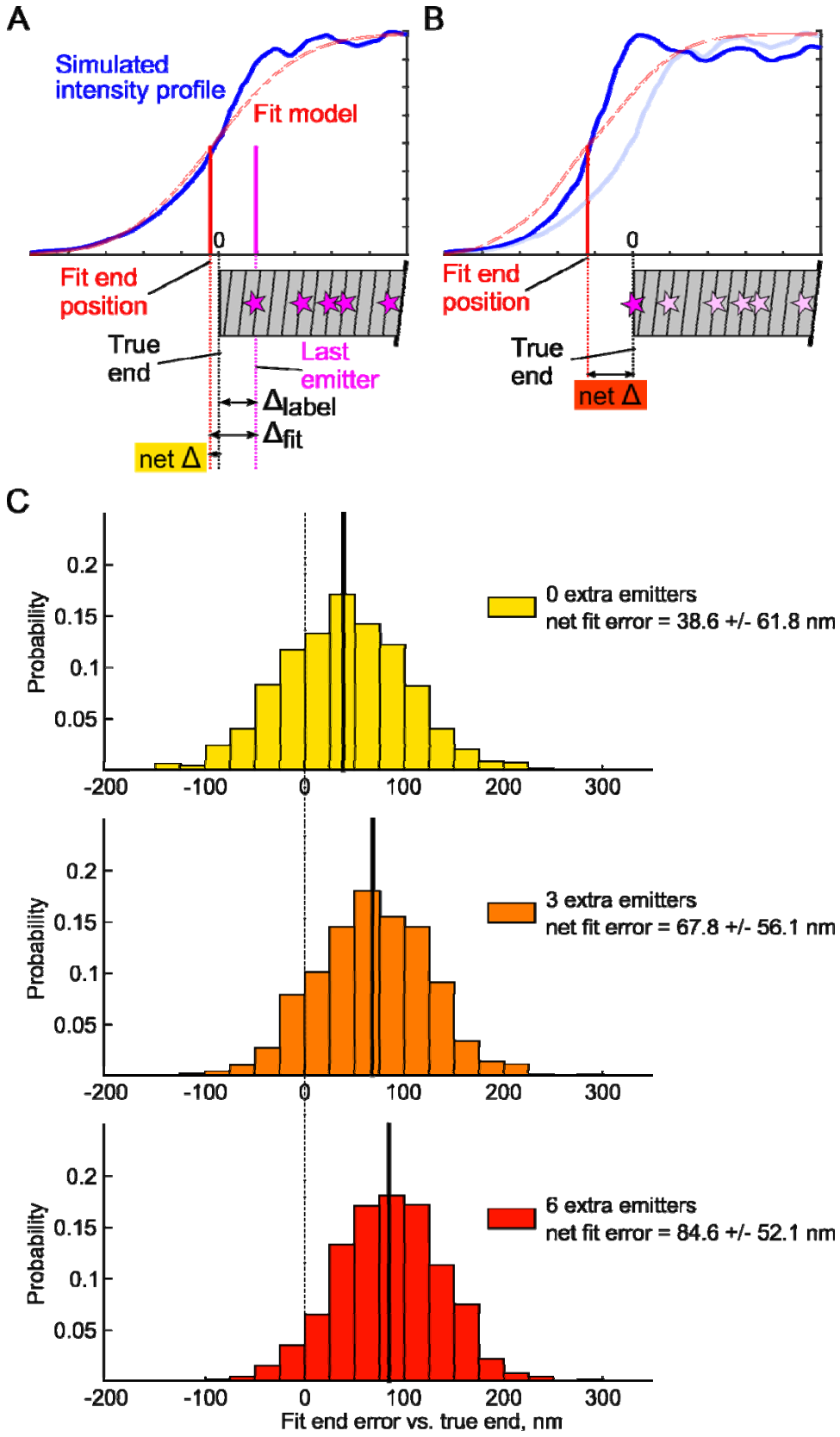
Supplemental Material

Supplemental Figures S1, S2, S3
Supplemental Table S1
Supplemental Movie S1
Supplemental Files: ImageJ and Matlab scripts

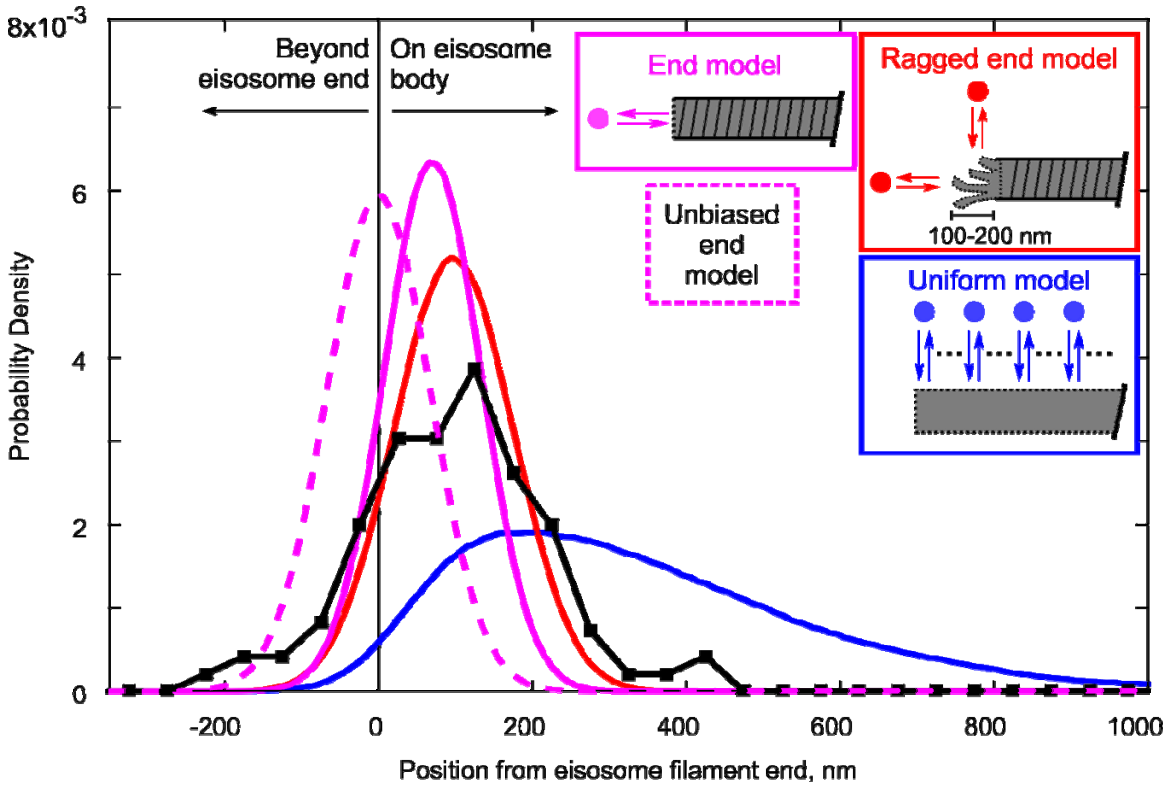
Supplemental Figures



Supplemental Figure S1. SNAP labeling of Pil1p in live fission yeast cells. (A-B) *S. pombe* cells expressing Pil1p-SNAP (A) or wild-type cells (B) were incubated with SNAP-SiR647 at indicated concentrations in EMM5S media for various times, washed and imaged in TIRF. The boxed panel highlights the sample condition used for further imaging and analysis, 15 hours at 0.5 µM SNAP-SiR647. (C-D) *S. pombe* cells expressing Pil1p-SNAP (C) or wild-type cells (D) were incubated with SNAP-Alexa647 at indicated concentrations in EMM5S media for various times, washed and imaged in TIRF. Images shown are inverted contrast, maximum intensity projections of 20-sec movies with median-filter background subtracted. Cell outlines are drawn in orange dash. All image panels are at same scale with scale bar 5 µm and same brightness scale.


A
Simulated intensity profile
Fit model
0
Fit end position
True end
Last emitter
Δlabel
Δfit
net Δ
B
0
Fit end position
True end
net Δ
C
Probability
0.2
0.15
0.1
0.05
-200
-100
0
100
200
300
0 extra emitters
net fit error = 38.6 +/- 61.8 nm
3 extra emitters
net fit error = 67.8 +/- 56.1 nm
6 extra emitters
net fit error = 84.6 +/- 52.1 nm
Fit end error vs. true end, nm

Supplemental Figure S2. Characterization of errors in simulated eisosome end localizations due to sparse labeling. (A) Schematic of errors in fitting: mock fluorescence intensity traces (blue) were generated by simulating a number of emitters uniformly distributed on a 350-nm region and then fit with the error function model (red line). Δ_{label}, distance between the last emitter (closest to the eisosome end) and the eisosome end; Δ_{fit}, distance between the eisosome end position estimated by fitting the fluorescence intensity and the last emitter; Δ_{net}, difference between the eisosome end position estimated by fitting the fluorescence intensity and the true end position. (B) Recruitment of new fluorescent molecules only at the filament ends introduces a systematic error in fitting. Mock fluorescence intensity traces (blue) were generated by simulating a number of emitters in a 350-nm region with one or more additional emitters at the eisosome end, and then fit with the error function model (red). Δ_{label} is reduced to zero and the intensity profile is skewed beyond the true eisosome end position. (C) Distributions of the differences between the fitted end and the true end (Δ_{net}) for simulated eisosome traces according to 3% labeling fraction with zero (yellow, 38.6 $\pm$ 61.8 nm), three (orange, 67.8 $\pm$ 56.1 nm), or six (red, 84.6 $\pm$ 52.1 nm) extra emitters added to the eisosome end (N = 1,000 simulations for each case). For each plotted distribution, the mean is shown as a black line, with the true end position shown as black dashed line. In all plots, the sign of $\Delta$is given as the effect on the calculated spot position (since estimating the end to be past the true structure causes the calculated SRAP spot position to be shifted toward the filament interior, a positive value).



Supplemental Figure S3. Alternate model for eisosome recovery dynamics. Probability distributions of measured distances (black squares, N = 191 spot/filament pairs across 20 cells) and simulation results (similar to Figure 3, N = 275,000 runs for each tested model). An additional "ragged end" model is shown (red), where simulations used simple Gaussian noise for the eisosome end position and calculated spot positions uniformly distributed within a discrete zone at the eisosome end. Simulated end model with biased end error (solid magenta) and unbiased end error (dashed magenta), uniform model (blue), and measured dataset (black) are as shown in Figure 3. Several values for the ragged end model zone were tested (not shown) to find an average position of 97 $\pm$ 76 nm for a 125-nm zone, similar to the measured data (97 $\pm$ 119 nm).

Table S1: *S. pombe* strains used in this study

Strain	Genotype	Source
FY527	*h- ade6-M216 his3-D1 leu1-32 ura4-D18*	S. Forsburg
JB198	*h- pil1-SNAP-kanMX6 ade6-M216 his3-D1 leu1-32 ura4-D18*	This study
JB204	*h- pil1-mEGFP-kanMX6 ade6-M216 his3-D1 leu1-32 ura4-D18*	This study
JB57	*h+ fim1-mEGFP-natMX6 ade6-M210 his3-D1 leu1-32 ura4-D18*	(Berro and Pollard, 2014)

Supplemental Movie S1

Cells expressing Pil1p-SNAP were labeled with SNAP-SiR647 at 0.5 µM for 15 hours, washed and imaged in TIRF. Movie was recorded at a single focal plane near the cell base at 10 frames per second. This movie file was used to generate parts of Figure 1 and Supplemental Figure S1. Scale bar 5 µm.

Supplemental Files 1 and 2: Scripts for image analysis and quantification

File 1: ImageJ Macro for finding, measuring recovery events and tracing eisosome ends: Supplement_script_ij.txt

File 2: Matlab script for fitting of eisosome filament ends, calculation of distance from SRAP spots to eisosome ends, simulations of eisosome end intensity profiles, and simulations of eisosome dynamics models: Supplement_script_matlab.txt

The results tables saved from ImageJ macro analysis can be opened in Excel to edit columns and clean to remove headings. The results tables for each movie file are copied into a separate Matlab file that loads the results tables as multidimensional arrays, called f[] and c[], and the results of the GDSC SMLM PeakFit plugin, called smlm[].
A separate results file is needed containing line measurements of eisosome lengths, manually drawn in AVG1-5 projections from all movies, total[].