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Supporting Material

Mechanical Distortion of Single Actin Filaments Induced by External Force: Detection by Fluorescence Imaging

Togo Shimozawa and Shin’ichi Ishiwata

Supplementary:

Movie S1 The fluorescence image of a single actin filament labeled with TMR at Cys^{374} obtained under the application of the external force in a rectangular waveform, alternately switching between ~5 and ~20 pN, which corresponds to Figs. 2 and 3A. The fluorescence intensity was linearly scaled in pseudocolor as shown in Figs. 2 and 3. The four white pixels shown in the first frame of the video indicate the corners of the rectangular area, where the fluorescence intensity was averaged. The movies (S1, S2, and S3) are the combination of the phase-contrast (left) and the fluorescence (right) images, which were captured simultaneously. The pixel size was 65.8 nm for the phase-contrast and 92.6 nm for the fluorescence image. The position of the upper and lower beads in the phase-contrast image (left) was adjusted to be right next to the same bead in the fluorescence image (right).

Movie S2 The fluorescence image of a single actin filament labeled with BODIPY at Cys^{374} obtained under the application of the external force in a rectangular waveform, alternately switching between ~5 and ~20 pN, which corresponds to Fig. 3B. The fluorescence intensity was linearly scaled in pseudocolor as shown by a color palette in Figs. 2 and 3.

Movie S3 The fluorescence image of a single actin filament labeled with rhodamine-phalloidin (RP-actin) obtained under the application of the external force in a rectangular waveform, alternately switching between ~5 and ~20 pN, which corresponds to Fig. 3C. The fluorescence intensity was linearly scaled in pseudocolor as shown by a color palette in Figs. 2 and 3.

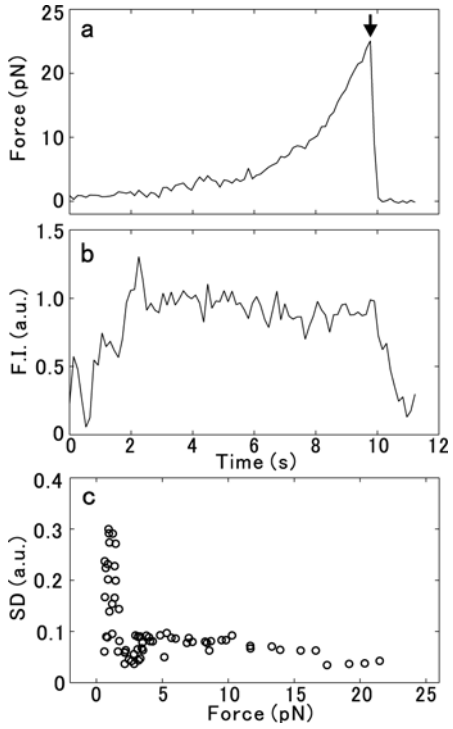

Figure S1 The relationship between the applied force, the fluorescence intensity, and the standard deviation (SD) of the fluorescence intensity fluctuation for RP-actin. As shown in Movie S3, the RP-actin filament was gradually stretched by moving the manipulated trap center, and the applied force (a) and the fluorescence intensity (b) were measured. The arrow in (a) shows the moment at which the filament was severed at the filament-bead interface. The data in (b) are shown with compensating for photobleaching. (c) The relationship between the SD (obtained from the data for 33 ms x 24 = 792 ms in (b)) and the applied force (corresponding to its value in the center of the same time interval in (a)).

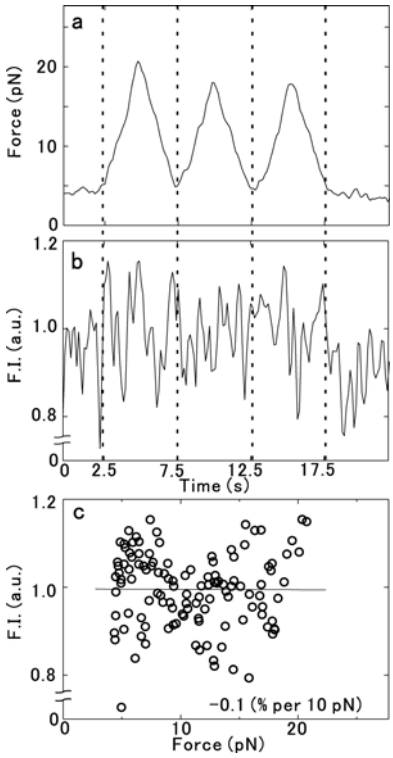

Figure S2　The effect of the external force, imposed in a triangular waveform, on the fluorescence intensity of QD-actin filaments. Actin filaments were labeled with quantum dots. The data analysis was done as in Fig. 4 except that the effect of photobleaching on the fluorescence intensity was not taken into account.