METHODS

Construction of E. coli Expression plasmids.

The circularpermutaion of YFP (cpYFP) was obtained by following the protocol as previously reported (1). The cDNA coding YFP was inserted into a pET-T7 vector, which a bacterial plasmid. The 1-144 a.a. was removed by inverse PCR and reinserted at XhoI site after 238 a.a. The NcoI site was newly generated at the site of 144 a.a. And then the primer including GGSGGT fragments was inserted between 238 and 1 a.a by inverse PCR. The GEWTYDD and TKTFTVTE fragments was made by annealing the primer coding each fragment, and inserted them at the NcoI and XhoI site, respectively (cpYFP-β1).

Kinesin constructs were derived from the original Drosophila cDNA clone according to the method by Yang et al (2). The cDNA clone of DK351 (amino acids 3-351) was inserted into the E. Coli expression vector pGEX-4T-1 (General Electric Healthcare Bio-Science Corp, NJ, USA) at the XhoI site, as previously described (3). DK351 was constructed with glutatione S-transferase (GST) fusion protein at the N-terminus, and BDTC at the C-terminus (DK351-BDTC). The cpYFP-β1 was inserted into DK351-BDTC at the AvrII site by using In-Fusion PCR cloning kit (Takara bio, Inc, Japan). The procedure of kinesin expression and purification is previously described (3). The DK351-cpYFP-β1-BDTC and DK351-YFP-BDTC were purified by a glutathione-agarose column. GST was then removed by proteolysis with thrombin. The final concentration of DK351-cpYFP-β1-BDTC and DK351-YFP-BDTC were 0.4 mg/ml and 0.5 mg/ml, respectively.

Absorbance and fluorescence spectra

The YFP, cpYFP, cpYFP-β1, cpYFP-β2 and cpYFP-β3 proteins were diluted to 0.01-0.1 mg/ml in 20 mM Hepes-NaOH (pH 8.0), and then scanned for absorbance between 250 and 600 nm (Shimadzu UV-Vis Spectrophotometer UV-1650PC). Fluorescence measurements were carried out on a Hitachi F-2700 Fluorescence spectrophotometer with a protein concentration of 0.1-0.3 mg/ml in 20 mM Hepes-NaOH (pH 8.0). The excitation wavelength was set to 488 nm and band width was set to 5 nm. The emission was scanned between 500 and 650 nm. For excitation spectra measurement, the emission wavelength was set to 530-550 nm. The excitation was scanned between 400 and 525 nm.

Model experiment of strain sensing by using kinesin recombinant

Fluorescence measurements were carried out on a Hitachi F-2700 Fluorescence spectrophotometer. The concentration of DK351-cpYFP-β1-BDTC and DK351-YFP-BDTC were prepared to 1 μM in a solution containing 20 mM K-PIPES (pH 6.8), 10 mM K-acetate, 4 mM $MgSO_4$, 2 mM EGTA, 0.2 mM EDTA, 0.2 mg/ml casein, and 40 μM Taxol. For excitation spectra measurement, the emission wavelength was set to 530-550 nm. The excitation was scanned between 400 and 525 nm. When adding microtubule, streptavidin and ATP, the final concentrations of them were 2μM, 0.001 mg/ml

and 4 mM, respectively. The measurements were done 5 minutes after the each addition.

REFERENCES

1. Nagai, T., Sawano, A., Park, E. S. & Miyawaki, A. (2001) Circularly permuted green fluorescent proteins engineered to sense Ca2+. *Proc Natl Acad Sci USA*. 98, 3197-3202.
2. Yang, J. T., Saxton, W. M., and Goldstein, L. S. (1988) Isolation and characterization of the gene encoding the heavy chain of Drosophila kinesin, *Proc Natl Acad Sci USA*. 85, 1864-1868.
3. Inoue, Y., Toyoshima, Y. Y., Iwane, A. H., Morimoto, S., Higuchi, H., and Yanagida, T. (1997) Movements of truncated kinesin fragments with a short or an artificial flexible neck, *Proc Natl Acad Sci USA*. 94, 7275-7280.

SUPPLEMENTAL FIGURE

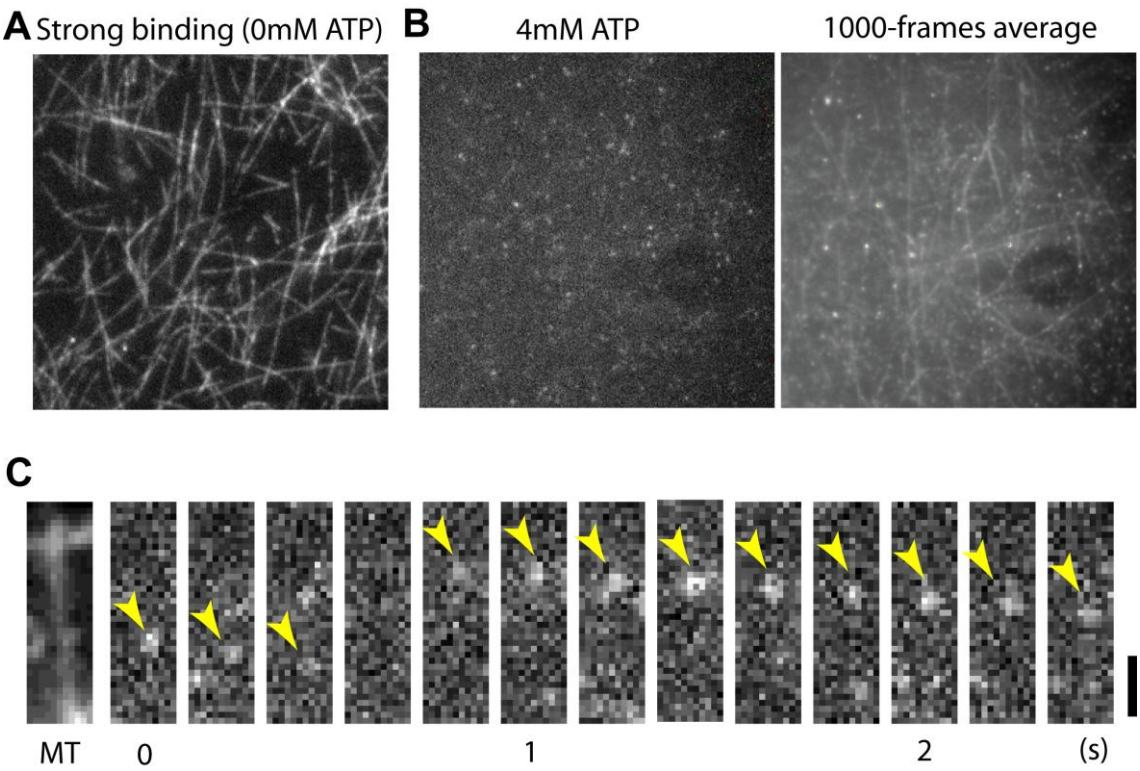

Fig. S1. Singe molecular assay of complex of DK351-YFP- BDTC and avidin.

The methods are shown in previous report (*). The buffer condition was 20 mM K-PIPES (pH 6.8), 10 mM K-acetate, 150 mM KCl, 4 mM $MgSO_4$, 2 mM EGTA, 0.2 mM EDTA, 0.2 mg/ml casein, 40 μM Taxol, and 1 % β- mercaptoethanol. 1 μM Kinesin and 0.001 mg/ml streptavidin were mixed and incubated for 10 min at 4 ^{o}C before the assay. The final concentration of kinesin was 1 nM in assay. (A) Fluorescent image of microtubules labeled with the complex of DK351-YFP-BDTC and avidin without ATP. (B) Fluorescent image of the complex of DK351-YFP-BDTC and avidin with 4 mM ATP. Left panel is a snap shot for 0.1 s. Right is an average image with 1000 images. Since DK351-YFP-BDTC specifically binds to and dissociates from microtubule, the microtubules appeared in the average image. (C) Processive movement of the complex of DK351-YFP-BDTC and avidin with 4 mM ATP. Yellow allows indicate moving DK351-YFP-BDTC.

(*)Watanabe TM, Iwane AH, Yanagida T, Single molecular observation of self-regulated kinesin motility (2010) *Biochemistry*, **49**, 4654–4661.

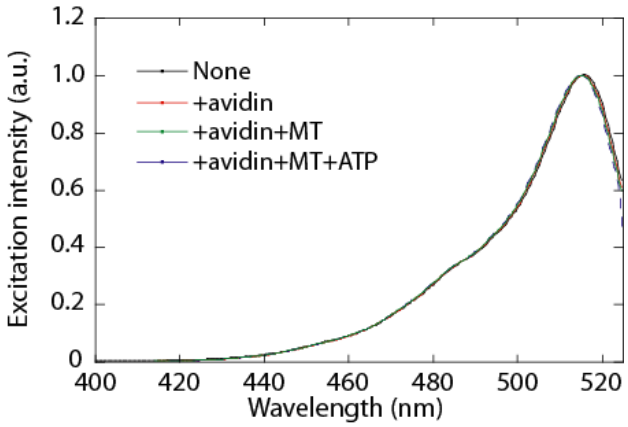

Fig. S2. Model experiments for strain sensing by using YFP.

Excitation spectrum of DK351-YFP- BDTC dimer at 50 mM K-acetate. We measured the excitation spectrum in the solution including nothing (black), avidin (red), avidin and microtubules (green), and avidin, microtubules and ATP (blue). The fluorescent intensities were observed from 530 to 540 nm. All intensity profiles are standardized at the each spectra peak.

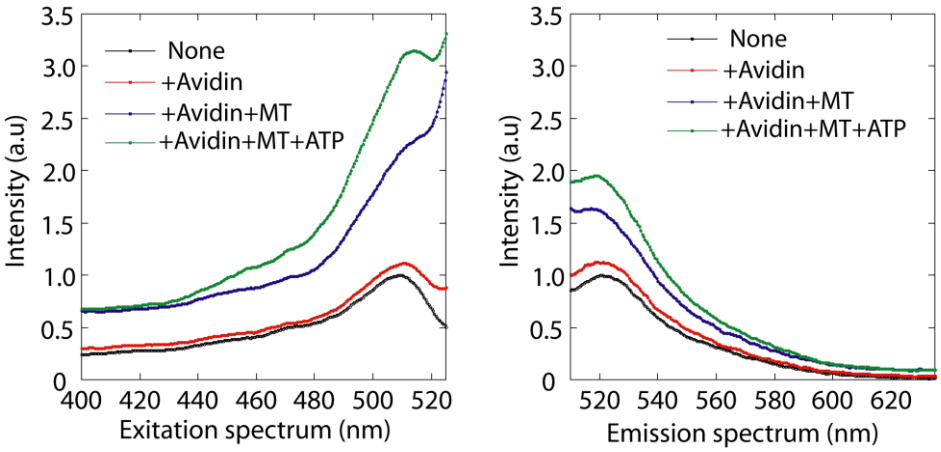

Fig. S3. Intensity change of DK351- cpYFP-β1- BDTC in model experiments for strain sensing.

Excitation (left) and emission (right) spectrum of DK351- cpYFP-β1- BDTC dimer at 50 mM K-acetate. We measured the excitation and emission spectrum in the solution including nothing (black), avidin (red), avidin and microtubules (green), and avidin, microtubules and ATP (blue). The fluorescent intensities were observed from 530 to 540 nm to obtain the excitation spectrum. The excitation wavelength was 488 nm in the emission spectrum. All intensity profiles are standardized at the spectra peaks in the solution including nothing.

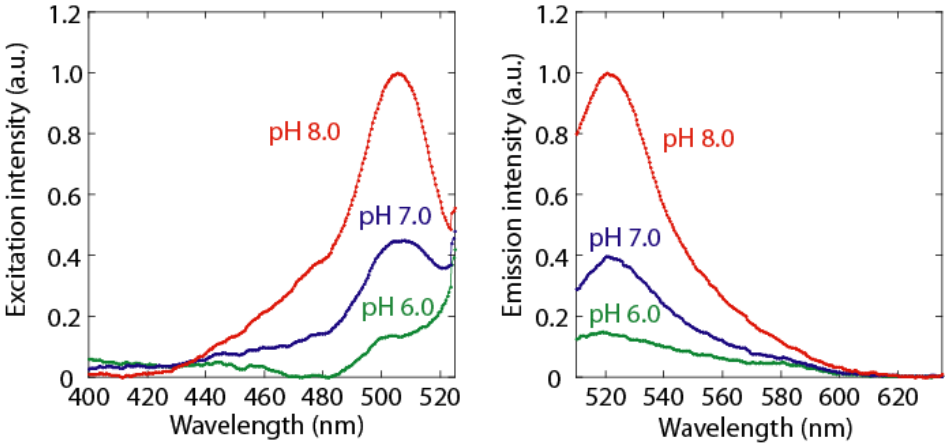

Fig. S4. pH dependency of excitation and emission spectrum of DK351- cpYFP-β1- BDTC.
Absorbance spectra of DK351- cpYFP-β1- BDTC in pH 8.0 (red), pH 7.0 (blue) and pH 6.0 (green). The fluorescent intensities were observed from 530 to 540 nm to obtain the excitation spectrum. The excitation wavelength was 488 nm in the emission spectrum. All intensity profiles are standardized at the spectra peaks in pH 8.0.

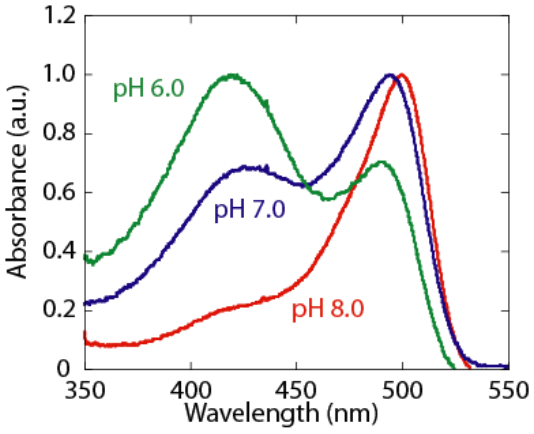

Fig. S5. pH dependency of absorbance spectrum of cpYFP-β1.
Absorbance spectra of cpYFP-β1 in pH 8.0 (red), pH 7.0 (blue) and pH 6.0 (green). The fluorescent intensities were observed from 560 to 570 nm. All intensity profiles are standardized at the each spectra peak.

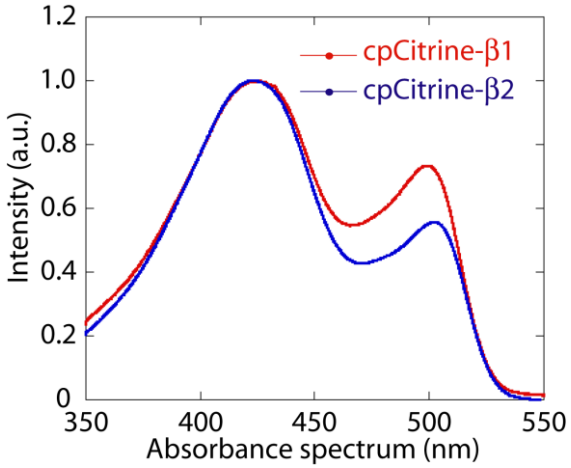

Fig. S6. Fluorescent characters of cpCitrine fused with β-hairpin.

Absorbance spectra of cpCitrine-β1 (red), cpCitrine-β3 (blue). "Citrine" means a recombinant YFP with Q69M mutation. The fluorescent intensities were observed from 530 to 540 nm. All intensity profiles are standardized at the each spectra peak.