Data Article

Myosin V fluorescence imaging dataset for single-molecule localization and tracking

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ARTICLE INFO

ABSTRACT

Myosin-5B is one of three members of the myosin-5 family of actin-based molecular motors fundamental in recycling endosome trafficking and collective actin network dynamics. Through single-molecule motility assays, we recently demonstrated that myosin-5B can proceed in 36-nm steps along actin filaments as single motor. By analyzing trajectories of single myosin-5B along actin filaments we showed that its velocity is dependent on ATP concentration, while its run length is independent on ATP concentration, as a landmark of processivity.

Here, we share image stacks acquired under total internal reflection fluorescence (TIRF) microscopy and representative trajectories of single myosin-5B molecules labelled with Quantum Dots (QD-myo-5B) moving along actin filaments at different ATP concentrations (0.3−1000 μM). Localization of QD-myo-5B was performed with the PROOF software, which is freely available [1]. The data can be valuable for researchers interested in molecular motors motility, both from an experimental and modeling point of view, as well as to researchers developing single particle tracking algorithms. The data is related to the research article "Dissecting myosin-5B mechanosensitivity and calcium regulation at the single molecule level" Gardini et al., 2015.

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Specifications table

Subject area	*Biophysics*
More specific subject area	*Single molecule imaging*
Type of data	*Fluorescence images, Tiff and graph*
How data was acquired	*Total internal reflection fluorescence (TIRF) microscope*
Data format	*Raw and analysed*
Experimental factors	*Recombinant HMM myosin-5B was biotinylated at its C terminal. Streptavidin-QDs (emission ~655 nm) were selectively attached to biotinylated myosin-5B at a 1:5 M ratio to ensure that a QD was conjugated to a single motor* [3]. *F-actin filaments where polymerized, labelled with rhodamine-phallodin (emission ~580 nm) and immobilized on the coverslip surface. No pretreatment of data*
Experimental features	*Image stacks were acquired with an EMCCD camera at 50–150 ms integration times, EM gain = 300 under TIRF illumination. Pixel size was 91 nm. Moving QD-myo-5B were isolated from immobile ones by visual inspection and regions of interest were cut from the original stacks to speed up the localization analysis*
Data source location	*Sesto Fiorentino, Italy, LENS - European Laboratory for Non-linear Spectroscopy*
Data accessibility	*All data is with this article*
Related research article	*Gardini et al. "Dissecting myosin-5B mechanosensitivity and calcium regulation at the single molecule level", Nature Communications 9, 2844 (2018)*

Value of the data

- Single molecule techniques allow tracking the movements of molecular motors with nanometer accuracy to investigate their mechanochemical and biophysical properties [4–6]. Here we share raw image stacks of QD-labelled myosin-5B molecules while they move on actin filaments immobilized on a coverslip surface. The data can serve as a reference to develop novel single particle tracking algorithms and analysis methods to precisely derive the position and movement of single molecular motors.
- Generation of molecular motor trajectories using our freely available tracking software (PROOF, available as supplementary material in Gardini et al. [1]) or different tracking methods [7,8] can provide a benchmark for testing molecular motor models and, in particular, walking models for the myosin-5 family of motor proteins [9–11].
- The shared data can be useful to test automated methods for detection of moving vs immobile particles, step detection methods, and trajectory fitting [12].

1. Data

The shared data are image stacks acquired with a single-molecule motility assay in which actin filaments were immobilized on a coverslip surface and single myosin-5B proteins, bound to a Quantum Dot (QD-myo-5B), were free to interact and possibly move along an actin filament in the presence of ATP (Fig. 1). Images were acquired under total internal reflection fluorescence (TIRF) microscopy, in which the sample is illuminated only in the vicinity of the coverslip surface. Table 1 lists the image stacks attached to this article in supplementary information, including ATP concentration and integration time. Measurements 0–12 are selected regions of interests, the FullField.tif stack contains 1000 frames of a full field (512×512 pixels) acquisition. We used the PROOF software described in Gardini et al. [1] to track the position of the QD-myo-5B and reconstruct QD x-y trajectories as described in the next section (Fig. 2). The trajectories were then used to compute myosin run length (the distance traveled by the motor before detaching from the actin filament) and velocity, listed in the last two columns of Table 1, as described in the next section.

2. Experimental design, materials, and methods

Single molecule motility experiments were performed on an inverted fluorescence microscope (Nikon ECLIPSE TE300) equipped with a 532 nm laser (Coherent Sapphire) for rhodamine excitation (~3 mW on the sample) and a 488 nm Laser Physics argon laser for QDs-655 excitation (~3 mW on the sample) [13].

The sample was loaded into a flow chamber of about 20 μL volume coated with 1% w/v nitrocellulose/amyl acetate on the coverslip surface. 2 mg/ml of inactivated NEM-myosin-2 was loaded into the
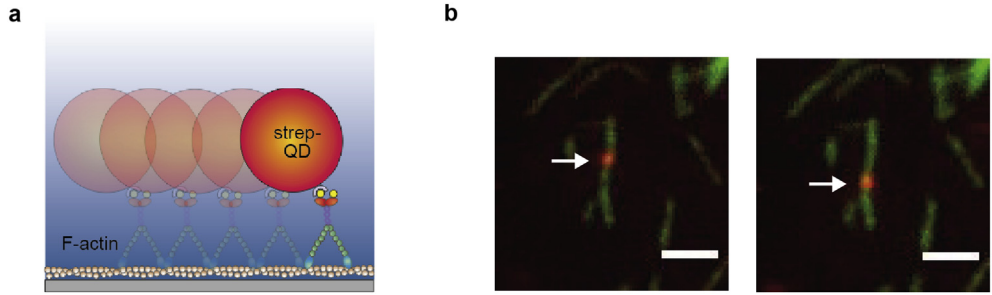
Fig. 1. a) Single molecule motility assay scheme. A QD-myo-5B is observed while proceeding along a single actin filament attached to a glass coverslip in the presence of ATP. **b)** Examples of QD-myo-5B motors moving along an actin filament (as indicated by the arrows). The images are superpositions of fluorescence images of actin filaments labelled with rhodamine phalloidin (green) and QD-myo-5B (red). Scale bar is 2 μm.

Table 1
Image stack number in supplementary materials is listed with the ATP concentration, the integration time of the measurement, the calculated run length and velocity.

measure	[ATP] (μM)	Integration time (ms)	Run length (nm)	Velocity (nm/s)
0	0.3	150	835	6
1	25	70	800	497
2	25	70	914	344
3	100	100	871	512
4	100	100	842	547
5	300	100	822	433
6	300	100	632	632
7	500	70	748	763
8	500	70	1077	699
9	700	70	854	718
10	700	70	995	618
11	1000	50	720	757
12	1000	50	625	781
FullField	100	100	–	–
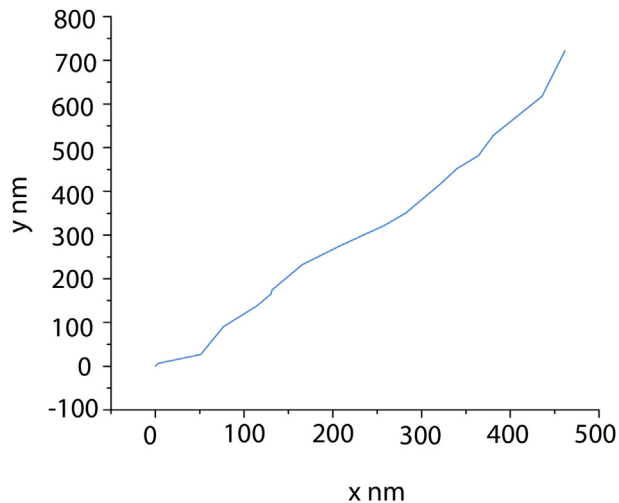
Fig. 2. Example of a trajectory obtained by x, y localization of QD-myo-5B at 100 μM [ATP] through the PROOF program. The trajectory is calculated from attached stack 3 (Table 1) and the coordinates values of this trajectory are listed in the supplementary table.

flow chamber and incubated for 1 min, followed by rhodamine phalloidin F-actin. After careful washing, 0.1 nM QD-myo-5B was introduced. Experiments were performed in the presence of 0.3−1000 µM ATP.

Images were acquired in TIRF microscopy using a Nikon Plan Apo TIRF, 1.45×NA, 60X oil immersion objective. The image pixel size was 91 nm, obtained by projecting the fluorescence image onto an iXon 3 EMCCD camera, after an additional 3 × magnification through an achromatic doublet telescope. Depending on the ATP concentration, different integration times (50−150 ms) were used at constant EM gain = 300 (Table 1) (see Gardini et al. [2] for detailed methods).

High precision localization of single QDs was performed by a custom-made Matlab algorithm, named "PROOF", that automatically detects the fluorescent emitter within a region of interest (ROI) and fits a bidimensional Gaussian function to its intensity profile [1]. Myosin trajectories were derived from x,y coordinates of QDs moving along actin filaments for more than ten frames. The run length was calculated from x−y trajectories (Fig. 2) [2].

Acknowledgments

Transparency document

Transparency document associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2019.103973.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2019.103973.

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