Measurement of the stall force of kinesins in living cells with *Lunam*™ T-40i by IMPETUX

Introduction

Motor proteins are responsible for different fundamental biological processes inside cells. One of these functions, of vital importance for the cell survival, is the intracellular transport of vesicles and organelles. In this convoluted scenario, molecular motors develop an important function by progressing along the filaments of the cytoskeletal network, delivering material at long distances, where transport through thermal diffusion becomes inefficient.

Kinesin is the microtubule-based protein that performs the plus-end-directed motion. The protein generates the mechanical work required to move cargos, by means of the hydrolysis of ATP molecules. The study of the mechanisms that govern its operation is crucial for a precise understanding of the big picture that represents transport in cells.

The analysis of these molecules has been possible by a fruitful symbiosis between different biophysical techniques. Among them, optical tweezers have played a central role in the study of the mechano-chemical properties of kinesin; the molecule, in turn, has become a gold standard in the biological applications of optical trapping.

The necessity to learn more about the physical interplay between motor proteins and other molecules present in cells during transport that are not considered *in vitro*, has forced optical trapping to move into this richer and more complex environment. Recent works have shown the measurement of forces on transported organelles and vesicles inside living cells [1-5].

One of the main problems in these measurements is the difficulty to obtain a reliable trap calibration inside cells. The measurement of forces is typically achieved by virtue of the linear relation between the applied force and the displacement of the sample in the trap, which results in the requirement of modeling the mechanical response of the sample under an external force to determine this trap stiffness calibration [6].

This represents a challenging task in the case of viscoelastic media or when bioactive processes are present, and has only been tackled recently [7]. However, despite these advances, the lack of a standard for measuring forces in such a complex medium still generates controversy on the reliability of the results. Discrepancies on the stall force of kinesin and dynein have been reported in the different works [1-5].

Lunam™ T-40i by IMPETUX

Impetux's *Lunam*™ T-40i (Fig. 1) incorporates a patented technology [8] that offers the possibility of determining forces when stiffness calibration is difficult or even impossible. After an accurate calibration at factory, the output signal is converted into units of force without the requirement of an *in situ* calibration. This is achieved by directly measuring the deflection of the laser as this goes through the sample, since, besides some constant factors, this directly corresponds to the optical force.

In addition, this more general approach makes the repeated calibration of the trap unnecessary, reducing the setting time and required expertise.

Fig. 1. *Lunam*™ T-40i sensor head.

The measurement-ready distinctive capability of *Lunam*™ provides a high-throughput instrument, which

allows for very efficient data recording of motor proteins' forces in cells.

Results

By stepping on the cytoskeletal substrate, kinesins can produce forces of some piconewtons. For loads of 6-7 piconewtons *in vitro*, the motor stops its motion and eventually disassociates from the filament until the thermally-driven oscillation of the cargo enables the protein to bind back to the microtubule. This corresponds to the stall force of the protein.

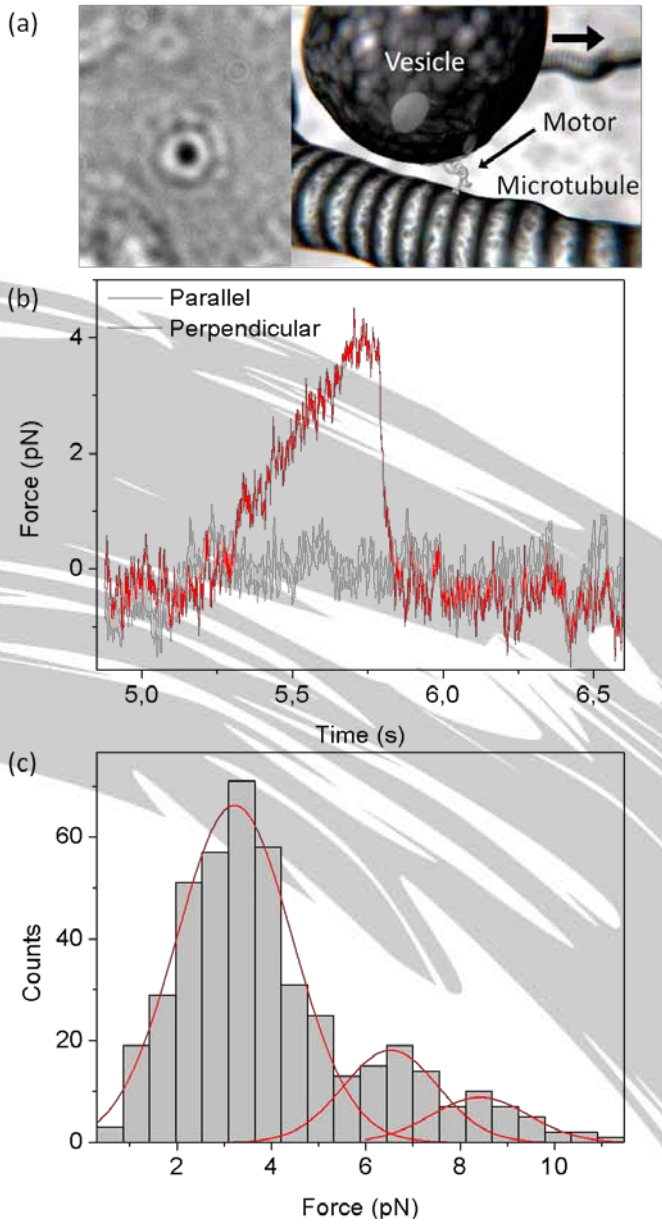

Fig. 2. (a) Image of a lipid droplet suspended in the cytoplasm of an A549 cell and simulation of a cargo transported by a motor protein along a cytoskeletal filament (the horizontal arrow indicates the direction of motion). (b) Typical stall force curve of a kinesin inside an A549 cell. The sampling frequency is 15 kHz. The two components of the force (parallel and perpendicular to the filament) are shown. The stepping capability of the protein decreases as the force generated by the trap approaches the stall force of the molecule close to 4 pN, which appears as a short plateau. The molecule suddenly detaches from the filament and the force drops to zero. (b) A histogram with some 400 positive events shows a peak at 3.2 pN and two additional peaks at multiples of this value.

Lipid droplets in A549 cells can be used as targets for trapping and analysis of the force of the motor proteins propelling them (Fig. 2a). Figure 2(b) shows the measurement with *Lunam*™ of the stall force of one of these lipid droplets moving in the outward direction. The direction of motion of the cargo (from the inner part of the cell towards de periphery) is indicative, in this cell line, of the action of a kinesin. When the experiment is repeated many times, the histogram of stall forces obtained with our instrument shows a main peak around 3 pN (Fig. 2(c)), in agreement with recent works [1-3].

Measurements like those shown here are an example of the potential of *Lunam*™ T-40i, which offers the possibility of measuring forces in traditionally difficult or impossible experiments. Furthermore, the instrument provides a remarkable ease of use for non-experts, both during installation and operation, and eliminates the time-consuming calibrations required for traditional systems. Moreover, uncertainties due to errors in previous calibrations are greatly reduced.

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