

## Measurement of the stall force of kinesins in living cells

### 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.

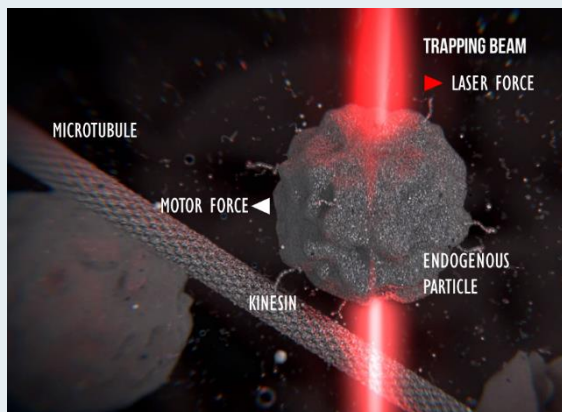


Fig. 1 - Schematic view of the optical trapping of a vesicle transported by a motor protein along a cytoskeletal filament.

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. 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. 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 [1]. This represents a challenging task in the case of viscoelastic media or when bioactive processes are present, and has only been tackled recently [2]. The lack of a standard for measuring forces in such a complex medium still generates controversy on the reliability of results.

### Results

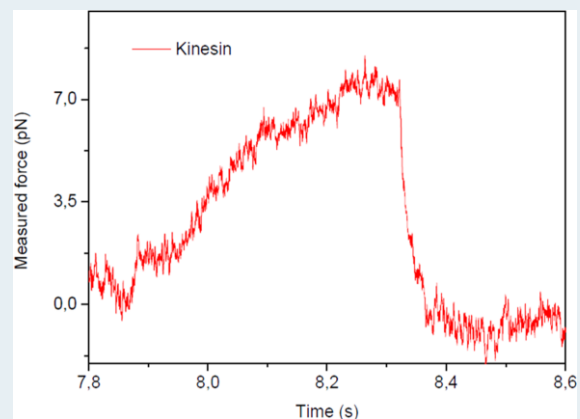
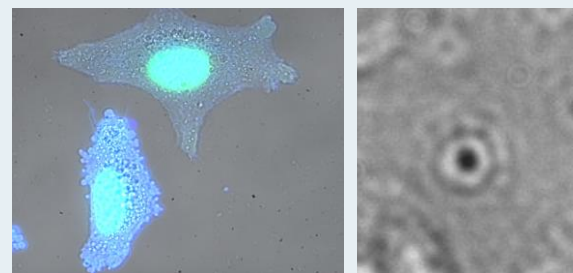


Fig. 2 - Image of a lipid droplet suspended in the cytoplasm of an A549 cell. Typical stall force curve of a kinesin inside an A549 cell. The stepping capability of the protein decreases as the force generated by the trap approaches the stall force of the molecule around 6-7 pN. The molecule suddenly detaches from the filament and the force drops to zero. The viscoelasticity of the medium is clearly seen as a slowing-down of the force relaxation after motor detachment.

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IMPETUX's **SENSOCELL** optical tweezers incorporate a patented technology [3] that offers the possibility of determining forces when trap stiffness calibration is difficult or even impossible, for example, in cells (Fig. 1).

By stepping on the cytoskeletal substrate, kinesins can produce forces of some piconewtons. In vitro, for loads of 6-7 piconewtons, 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 [4]. This corresponds to the stall force of the protein. In cells, lipid droplets can be used as targets for trapping and analysis of the force of the motor proteins propelling them. Figure 2 shows the measurement of the stall force of one of these lipid droplets in an A549 cell. The direction of motion of the cargo (from the inner part of the cell towards periphery) is indicative, in this cell line, of the action of a kinesin. The measured force is consistent with the results obtained by the pioneering groups with purified proteins and microspheres [4].

Measurement of forces inside cells allows exploring the rich interplay between multiple motor proteins simultaneously pulling on the same vesicle or

organelle. Figure 3 shows an example illustrating two opposed scenarios: cooperation and competition. Measurements like those shown here are an example of the potential of SENSOCELL in the motor protein field, which offers the possibility of measuring forces in traditionally difficult or impossible experiments. Furthermore, the distinctive technology used by the instrument makes the repeated calibration of the trap unnecessary and provides hence a remarkable ease of use for non-experts and eliminates the time-consuming calibrations required for traditional systems. Moreover, the measurement-ready distinctive capability of IMPETUX's technology provides a high-throughput instrument, which allows for very efficient data recording of motor proteins' forces in cells, and uncertainties due to errors in in situ calibrations are eliminated.

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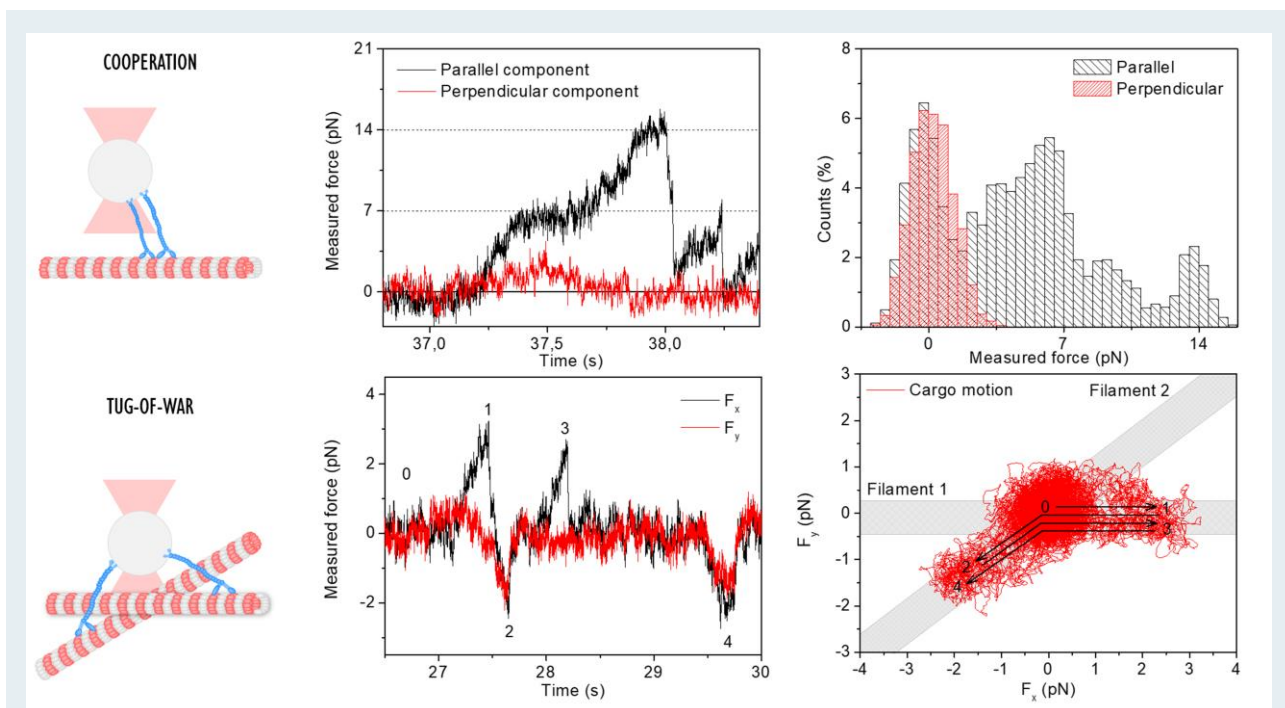


Fig. 3 - Force curves for a lipid droplet in an A549 cell pulled by multiple molecular motors in different scenarios: in cooperation and in competition. The two components of the force (parallel and perpendicular to the filament) are shown. In the first scenario, peaks in the histogram of forces show up at 0, 7 and 14 pN, indicating the sum of the stall forces of two kinesins simultaneously pulling on the same cargo in the same direction. In the second case, no plateaus at 7 pN (stall forces) are visible. The competition between the two motors moving along filaments with different directions is shown as a back-and-forth motion of the droplet.

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