Universidad Mayor para espíritus emprendedores | Vicerrectoría de Investigación CENTRO DE GENÓMICA Y BIOINFORMÁTICA | Mechano-Biology Group

Developing a Magnetic-Tweezers for the Study of Elastic Proteins

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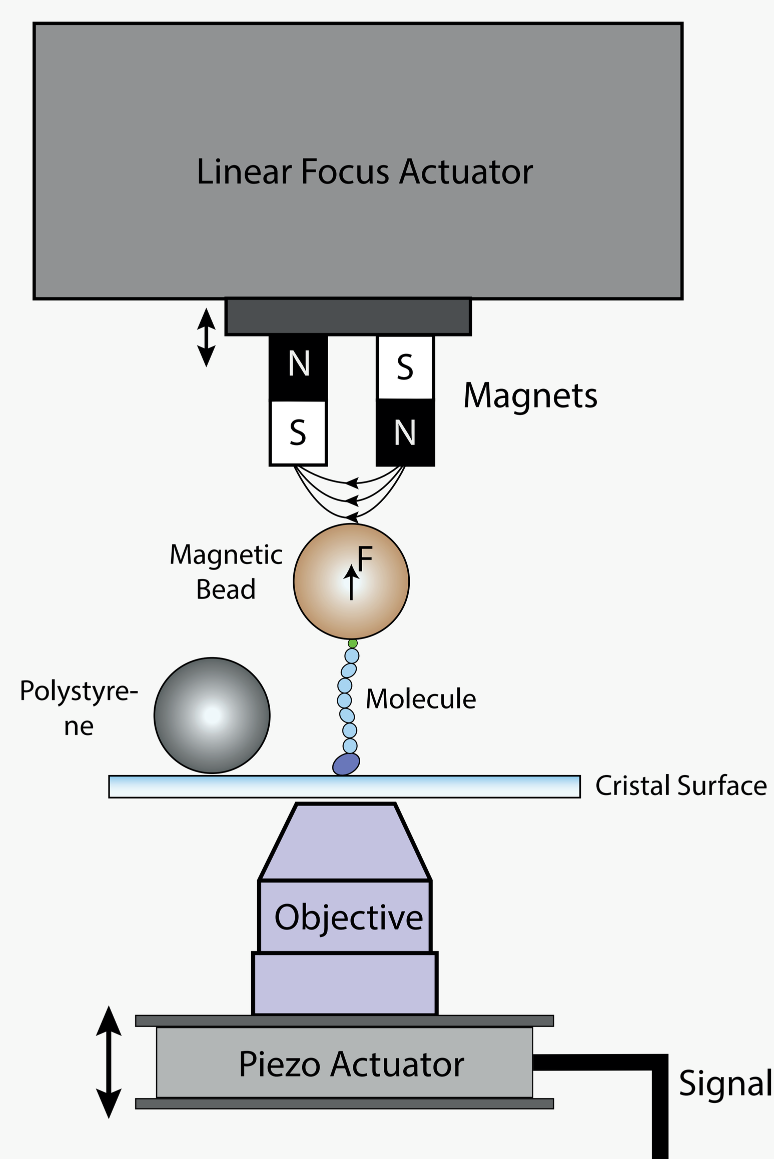
Introduction



Cell communicates through multiple stimuli, including electrical and biochemical cues. However, in the last decades, evidence has accumulated to suggest the appearance of the third kind of signal, mechanical cues, or forces that can be generated or transmitted by different molecular systems within the cell (1).

Dedicated instrumentation needs to be developed to study the effect of mechanical forces on biological processes, able to apply calibrated forces on protein molecules (2) (Figure 1). Here, we developed high-speed Magnetic Tweezers (MT) based on a lab-made inverted microscope, which includes 3D printed components and Open-Source Design and Software. For the calibration, we used protein L, a bacterial protein widely used as an archetype protein for folding experiments.

Figure 1. Mechanism of a Magnetic Tweezer. The MT is base on a magnetic field generated by a pair of magnets. Those attract a magneic beads attach to a molecule of interest. The molecule extension is measure by an objective and a piezo actuator and is interpreted by a PC.

Methods

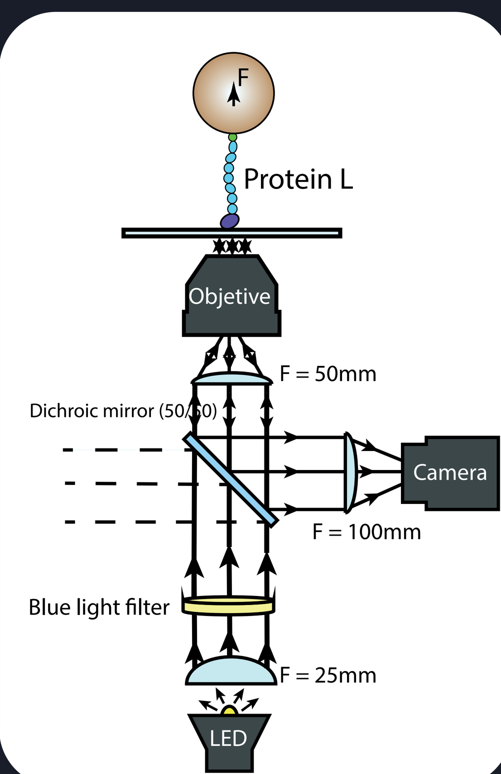


Figure 2. Microscope Light Path Design. Representative diagram of light-path for a conventional inverse microscope. The principal component is a dichoic mirror, that allows reflect 50% of light beams and the transmission of the other 50%. This mirror allow us to redirect light beam from the objective to the camera. For transming the light to objective we use convex-planar lenses with different focal lengths and also a blue light filter that preserve the integrity of the sample during the measuments.

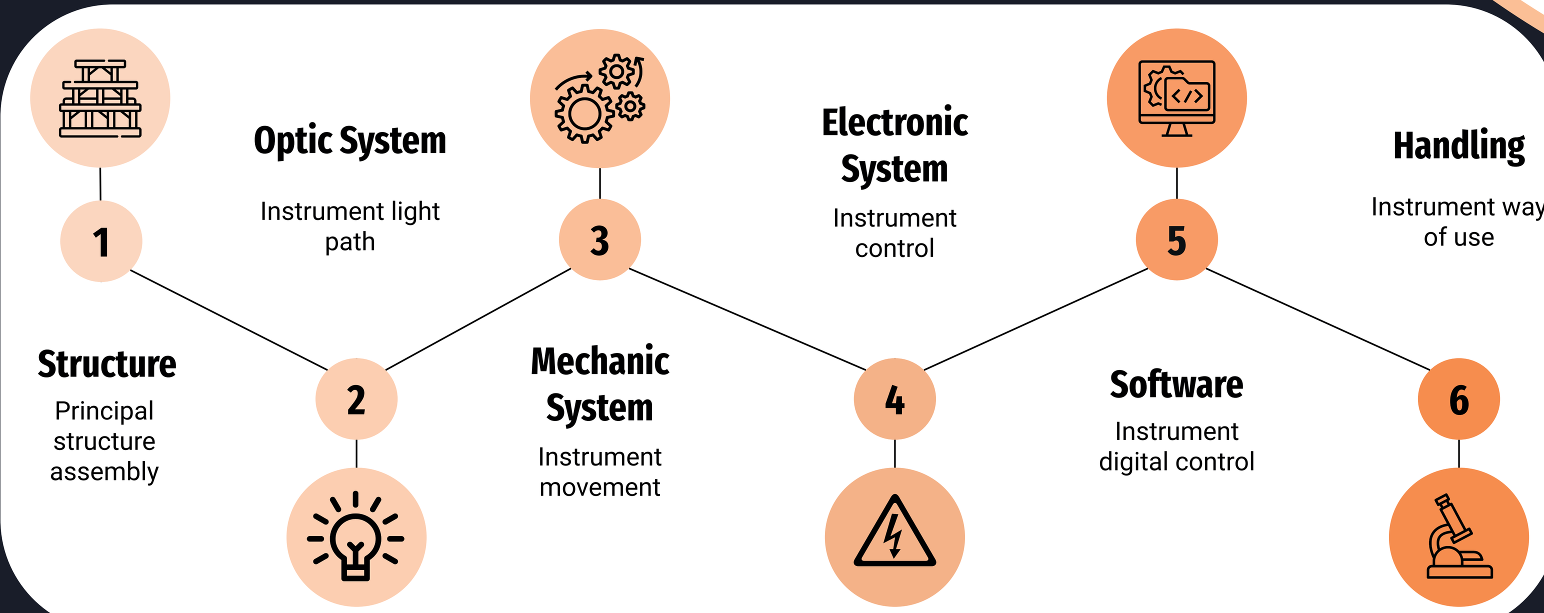
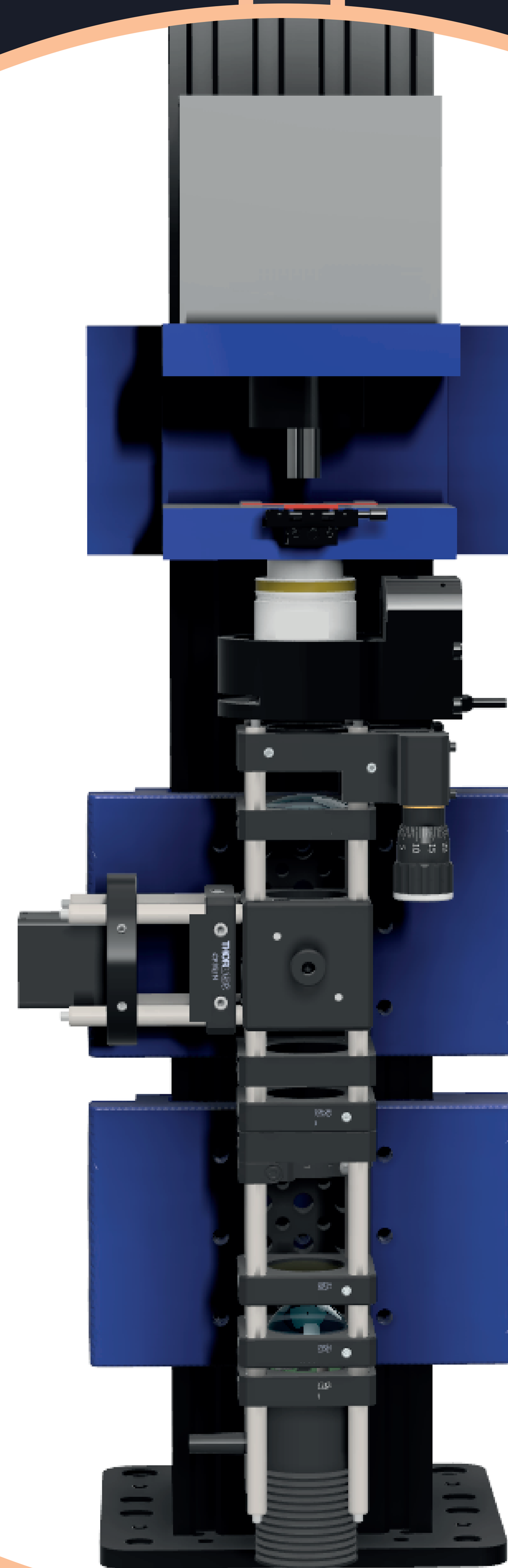


Figure 3. Methodology for Development of Magnetic-Tweezer. Schematic view of the steps to develop the instrument. First, we implement the chasis which allows the assembly of functional components of tweezers. Then, the optical-mechanical system were assemble; both work like a normal inverted microscope. On the top of the microscope, we mounted the linear-actuator, wich allow us the positioning of the magnetic pair. Finally, we control our instrument through a software, wich is available in GitHub.



Results

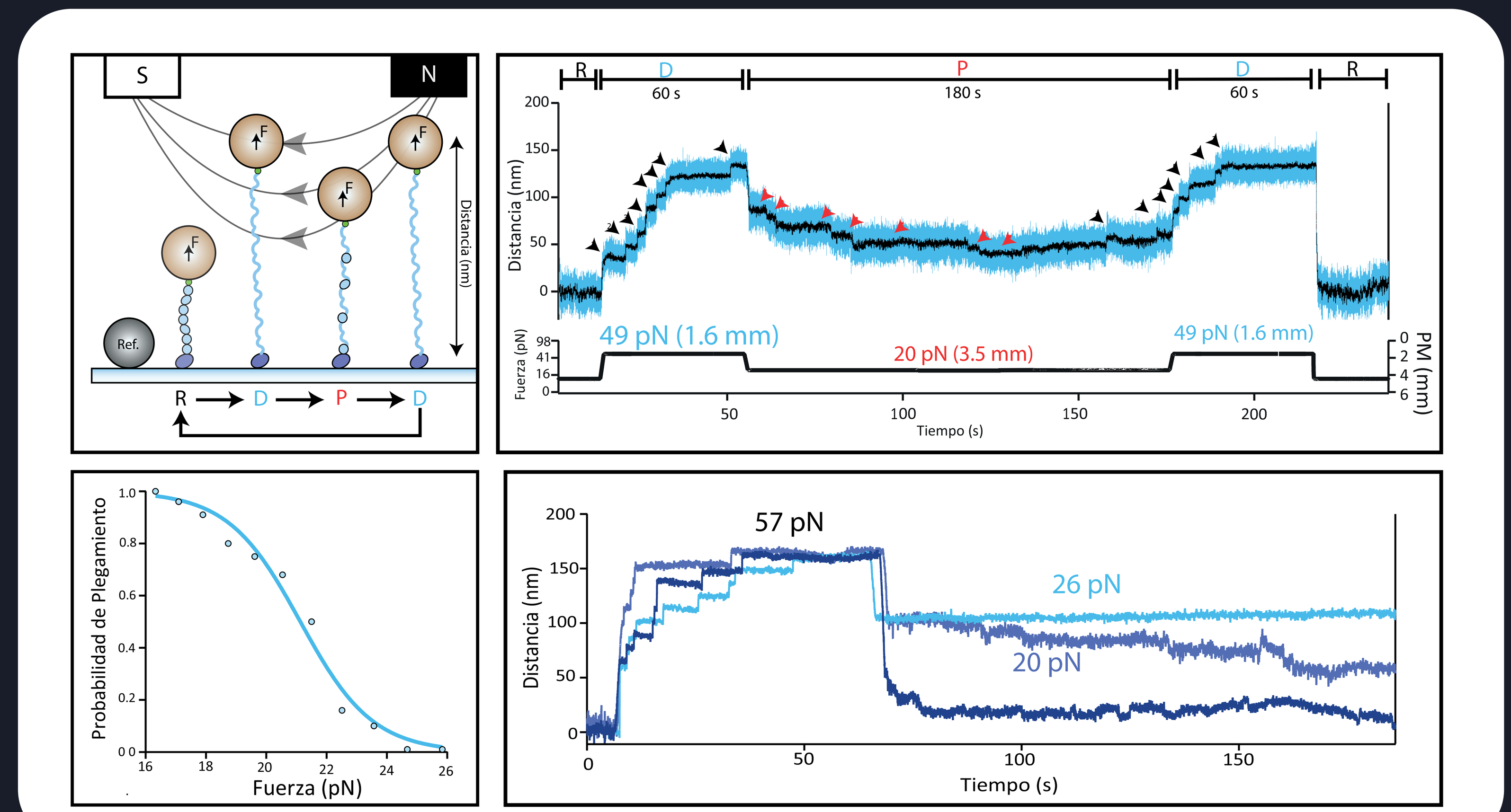


Figure 4. Folding and Unfolding of Protein L . Unfolding and refolding test using different pulses applied by a cycle protocol. A) Diagram illustrating the stages of the cycle of applied pulses. Beginning the test in a quiescent state (R), then follows an initial deployment pulse (D). After 60s, it decreases its strength, to allow retraction (P) and finally a final unfolding pulse (D'). Finally, the rest pulse is repeated, restarting the cycle. B) Representation of a complete un folding and refolding cycle of the L protein. In this event, a total of 8 domains were unfolded in the first pulse (black arrows). In the refolding pulse, 5 domains were folded, as observed (red arrows) and finally in the following unfolding pulse (D'), those 5 previously folded domains were unfolded, using the same force as in the first unfolding (49 pN). C) Refolding rate according to the events analyzed (total events > 100), where the number of refolded domains was divided by the total number of unfolded domains in the first pulse. Forces from 16 pN to 26 pN were used. D) Set of events carried out in folding pulses (P), with different applied forces.

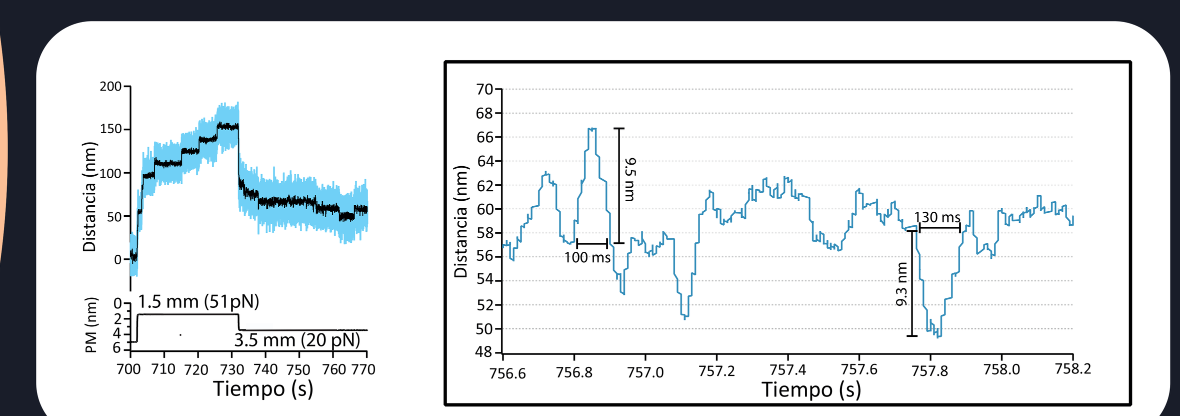


Figure 5. Determination of instrument error and its filtering. Comparison of data sets with and without Savitzky–Golay smoothing. A) Data set in the state of least force (7.5 pN), without application of smoothing. B) Data set in the state of greatest force (51 pN), without application of smoothing. C) Data set with the unfolding of a domain of the L protein at a force of 51 pN, without smoothing. D) Data set in the state of least force (7.5 pN), with smoothing applied. E) Data set in the state of greatest force (51 pN), with application of smoothing. F) Data set with the unfolding of a domain of the L protein at a force of 51 pN, with smoothing.

Conclusions

The MT setup yield recordings of single molecule within the milisecond time resolution, enable to study fast folding transitions in the range of 100 - 200 ms. On the other hand, we were also able to measured the rate of unfolding and refolding for the protein L, using a calibrated low-force in scale of 10-100 picoNewtons.

Furthermore, throught this project we could developed a low-cost instrument with a high spacio-temporal resolution, capable of tracking the mechanical behaviour of single protein molecules. By applying calibrated force is possible to access to the unfolded and folded state of the same protein during hours.

Finally, using 3D-printing and modular desing we managed to transform a high-cost instrument ($100.000) into a low-cost alternative ($20.000), including all the tools implemented to develop this MT. Also this instrument is the first of its kind (Magnetic Tweezers) here in Chile, with the ability of measure single molecula and single cell with an automatic software.

References

1. Jaalouk DE, Lammerding J. Mechanotransduction gone awry. Nat Rev Mol Cell Biol. 2009 Jan;10(1):63–73
2. Popa I, Rivas-Pardo JA, Eckels EC, Echelman DJ, Badilla CL, Valle-Orero J, et al. A HaloTag Anchored Ruler for Week-Long Studies of Protein Dynamics. J Am Chem Soc. 2016 Aug 24;138(33):10546–53.

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