THE AUTHOR FILE

Marco Capitanio

Tugging at single molecules shows how they shake hands.

When he set out to combine physics and biology, "there was no lab," says Marco Capitanio, a researcher at the University of Florence. "Someone loaned us a piece of an optical table." After finishing his master's degree in physics, he gave in to his long-standing interest in biology and obtained his PhD in physiology.

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He then joined the lab of Francesco Pavone, who was building a biophotonics program at the University of Florence within the context of LENS, the European Laboratory for Non-linear Spectroscopy. LENS is part of a European network of laser and spectroscopy facilities called Laserlab, a consortium of 26 research centers.

"LENS is a very nice place to work," says Capitanio, citing its interdisciplinary activities and scientists from around the world all using light to investigate an array of questions. "It's a stimulating environment." He is currently a researcher in Pavone's lab, where projects range from single-cell manipulation to neural imaging and biosensing.

Capitanio's work began after he bought a microscope and built optical tweezers to explore ways to measure molecular interactions with speedy unbinding kinetics. For example, myosin binds and moves actin in less than 1 millisecond. "This is very fast—too fast to be measured with the single-molecule techniques that were available," he says.

He and his colleagues applied a kind of optical leash to tug at the bonds between single molecules, thereby showing the dynamics of molecular bond formation and adding detail to other results generated with single-molecule force spectroscopy methods.

The researchers tested their dual-trap force-clamp configuration, which includes an optical microscope capable of fluorescence detection combined with double optical tweezers, to look at the interplay between actin and myosin in mouse skeletal muscle as well as the interaction between the bacterial lactose repressor protein and DNA. "The most interesting advancement in this method is the increased time resolution," Capitanio says.

Previous studies had shown that actin and myosin bind and unbind in two phases, but the team's data analysis revealed this interaction's third subchapter. Although models and extrapolations had indicated such a result, actual single-molecule measurements are new, Capitanio says.

For the myosin test, the researchers extracted a myosin head: in particular, a single molecular motor, the unit that produces movement and force. Both ends of an actin fiber were attached to individual beads in a dumbbell configuration and manipulated with optical tweezers.

Exerting a continuous load on the molecule called for a double-trap system with optical tweezers moving continuously and rapidly, thereby maintaining a constant force, says Capitanio. "The trap works like a spring." As the molecule moves in one direction and pulls on the spring, the forces change. "The trap has to move to maintain the spring length constant," he says.

To get ready for measurements, Capitanio wanted to apply force before the molecule bound to its partner, which was tricky. The solution was to alternate the direction of the force, moving back and forth and scanning until "we see some interaction," he says. "At some point, if you are in the exact position, the myosin molecule binds and you find it." At that binding moment, the system stabilizes and can take measurements.

In his next projects, he wants to study gene expression: for example, to measure protein conformational changes in the lac repressor system. He hopes other researchers will try his system, which uses standard optics components, but he says that a field-programmable gate array board is essential for acceleration. Dissecting the data output to find the molecular interactions was challenging. He is finalizing his software and its 10,000 lines of code to share with the community.

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Capitanio's technique has offered "unequalled frequency response," allowing measurement of mechanical as well as kinetic parameters of the actin-myosin interaction, says Vincenzo Lombardi, physiologist at the University of Florence and Capitanio's PhD advisor.

Young scientists have a challenging time in Italy, where positions are scarce, and competitive jostling can easily distract people from their research focus. Through his motivation and methodologically and conceptually gifted approach, Capitanio has opened new approaches in muscle biophysics, says Lombardi. "As a physiologist, I can especially appreciate his ability to face with the right attitude and determination the complexity of a biological problem."

Vivien Marx

Capitanio, M. *et al.* Ultrafast force-clamp spectroscopy of single molecules reveals load dependence of myosin working stroke. *Nat. Methods* **9**, 1013–1019 (2012).