

# Light used to measure the 'big stretch' in spider silk proteins

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While working to improve a tool that measures the pushes and pulls sensed by proteins in living cells, biophysicists at Johns Hopkins say they've discovered one reason spiders' silk is so elastic: Pieces of the silk's protein threads act like supersprings, stretching to five times their initial length. The investigators say the tool will shed light on many biological events, including the shifting forces between cells during cancer metastasis.

"All other known springs, biological and nonbiological, lengthen in a way that is directly proportional to the force applied to them only until they

have been stretched to about 20 percent of their original length," notes Taekjip Ha, Ph.D., the study's lead researcher. "At that point, you have to apply more and more force to stretch them the same distance as before. But the piece of the [spider silk protein](#) we focused on continues to stretch in direct proportion to the force applied until it reaches its maximal stretch of 500 percent."

Details of the research were published online in the journal *Nano Letters* on Feb. 5.

Ha, a Bloomberg Distinguished Professor of biophysics and biophysical chemistry at the Johns Hopkins University School of Medicine, says the new discovery came during follow-up to research he and his team, then at the University of Illinois at Urbana-Champaign, [described in the journal \*Nature\*](#) in 2010, work done in collaboration with cell biologists led by Martin Schwartz, then at the University of Virginia.

The Virginia team set up those experiments by inserting a repeating amino acid sequence—taken from the spider silk protein known as flagelliform—into a human protein called vinculin. Vinculin is responsible for internalizing forces outside a cell by bridging the cellular membrane and the actin network within the cell, making it an important mechanical communicator within the cell.

The scientists also flanked the flagelliform insert in vinculin with two fluorescent proteins to light up and "report" what was going on through fluorescence resonance energy transfer, or FRET. FRET occurs when one fluorescent molecule is close enough to another that it activates the second. So, when vinculin was relaxed within a cell, it "glowed" yellow, the color of the second fluorescent protein being activated by the first. As vinculin stretched, it began to glow blue—the color of the first fluorescent protein—because the lengthening distance between the two made FRET activation of the yellow protein impossible.

Using regular fluorescence microscopy, the scientists were able to watch the forces acting on vinculin in live cells in real time. But an issue remained: how to translate the changing colors into measurements of force "sensed" by vinculin.

That's where his team came in, says Ha. The researchers attached one end of modified vinculin to a glass plate and the other to a tether made of DNA with a small plastic bead at the end. They then pulled on the bead with what Ha describes as "chopsticks made of light," focusing a beam of light on a tiny spot nearby and generating an attractive force that pulled the bead toward the light source. That way, Ha says, his investigators could link the amount of FRET with the amount of force on vinculin, allowing them to measure the dynamic forces acting on proteins in [live cells](#) just by imaging them.

In that earlier study, the team inserted 40 flagelliform [amino acids](#) into vinculin, composed of eight repeats of the amino acid sequence GPGGA. In this new study, the scientists wanted to learn more about the flagelliform tool by varying its length, so they created inserts of five and 10 repeats to test alongside the original insert of eight. What they found is that the shortest insert was the most responsive to the widest range of forces, responding with linear increases in length to forces from 1 to 10 piconewtons. (Ha says that 1 piconewton is approximately the weight of a bacterium.)

The team wasn't expecting the spider silk inserts to show such linear behavior because, according to Ha, they don't form well-defined, three-dimensional structures. "Usually, unstructured proteins show disorderly, nonlinear behavior when we pull on them," says Ha. "The fact that these don't act that way means that they will be really useful tools for studying protein mechanics because their behavior is easy to understand and predict."

Already, Ha says, the flagelliform insert of eight repeats from the previous research has been used to study many biological phenomena, including the shifting forces between cells during [cancer metastasis](#) and the pushing and pulling of cells during the development of simple, multicelled organisms, like worms.

"Tension is important for many activities inside cells," says Ha. "Cells sense mechanical forces in their environments and change their behaviors and functions in response. Now we have a way to watch and understand these forces and how they are transmitted at a molecular level in living cells."

**More information:** Michael D. Brenner et al. Spider Silk Peptide Is a Compact, Linear Nanospring Ideal for Intracellular Tension Sensing, *Nano Letters* (2016). [DOI: 10.1021/acs.nanolett.6b00305](https://doi.org/10.1021/acs.nanolett.6b00305)

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