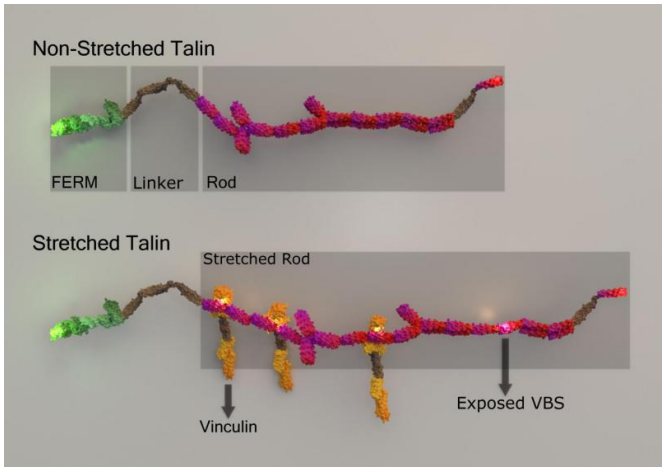
Multicolour super resolution imaging – A method to monitor dynamic protein binding at subsecond timescales

17 June 2016



Talin stretching and stretch-induced vinculin binding. Credit: National University of Singapore

Researchers from the Mechanobiology Institute (MBI) at the National University of Singapore have developed a new method, using super-resolution microscopy, to determine the length of stretched proteins in living cells, and monitor the dynamic binding of proteins, at sub-second timescales. This study was published in *Nano Letters* in May 2016.

Cells are constantly exposed to mechanical forces. These signals influence cellular decision making by providing information cells need to determine how much of a particular protein to produce, when a specific gene should be expressed, or even whether a cell should move or remain where it is. Such information is crucial, for example, in maintaining the health, integrity and repair of tissues as we age. A clear example of when cells are exposed to forces is when we walk. Stretching or pulling forces are generated within our muscles, and these are passed through the muscle to connective tissue and bone. Although this information is generated at a tissue level, it converges on single cells within those tissues, and is detected and measured by subcellular, protein based, machines.

To measure the forces applied to a cell, specialized proteins may be deformed. A common way that this occurs is when a protein is stretched, just like how an elastic band stretches when subjected to pulling forces. Stretching of proteins can expose regions within them that are otherwise hidden. These regions can serve as docking sites for the attachment of other proteins. This leads to a snowball effect, wherein more and more proteins are able to bind, and larger molecular complexes or machines form to mediate a specific cellular function. This phenomena was recently explored by MBI Director, Professor Michael Sheetz, Senior Research Fellow Dr Felix Margadant and PhD student Ms Xian Hu (Edna), in work focused on characterizing the stretching of a force-sensing protein known as talin, and establishing the effect it has on the binding of another protein called vinculin.

Although several studies have shown the force-induced stretching of talin and talin-vinculin binding in vitro, simultaneous visualization of both these events and their correlation to specific cellular functions was not previously possible in living cells due to the rapid time scales at which they occur. Also, carrying out multicolor super resolution imaging in living cells is still very difficult. To overcome these challenges, Prof Sheetz and Ms Hu developed a novel, and highly advanced super-resolution imaging method, that allowed them to simultaneously monitor talin length in living cells, as well as the dynamics of vinculin binding, at single molecule level and millisecond timescale.

By attaching different fluorescent molecules (GFP

and mCherry), to each end of the talin and a third fluorophore (Atto655) to vinculin, the researchers could monitor the precise subcellular location of each protein, and confirm that when talin was being stretched, vinculin bound to newly exposed sites. Interestingly, their findings often revealed clustered binding, with five or more vinculin molecules binding to talin in one second. Moreover, the binding of the first few vinculins seemed to energetically favor the successive binding of more vinculin molecules. Correlating vinculin binding dynamics with the amount of talin stretching, the researchers noted that maximum vinculin binding occurred at one specific end of talin (the N-terminal region), when talin was stretched to approximately 180 nm.

Understanding how talin and vinculin respond to stretching forces is crucial to understanding how cells respond to forces in our bodies. In this case, both proteins are found in larger molecular machinery called focal adhesions, which physically connect the interior of a cell with the material that is surrounding the cell, the extracellular matrix. Focal adhesions primarily function as signal relaying centers, and the information they transfer can induce cell growth and cell movement. When this signal processing is disrupted, or is not regulated, disease states arise and the body's ability to heal wounds, or maintain tissue integrity as we age becomes impaired.

Although important to facilitating these wider cellular and tissue processes, the talin-vinculin interaction is just one of many protein interactions to respond to force. It is hoped that this newly described method will pave the way for researchers to dissect other protein interactions, both within focal adhesions, and in other molecular machines, to improve our understanding of the many force-driven cellular processes that arise during development and continue through to aging.

More information: Xian Hu et al. Cooperative Vinculin Binding to Talin Mapped by Time-Resolved Super Resolution Microscopy, *Nano Letters* (2016). DOI: 10.1021/acs.nanolett.6b00650

Provided by National University of Singapore