Measuring the tension of a cell with a molecule

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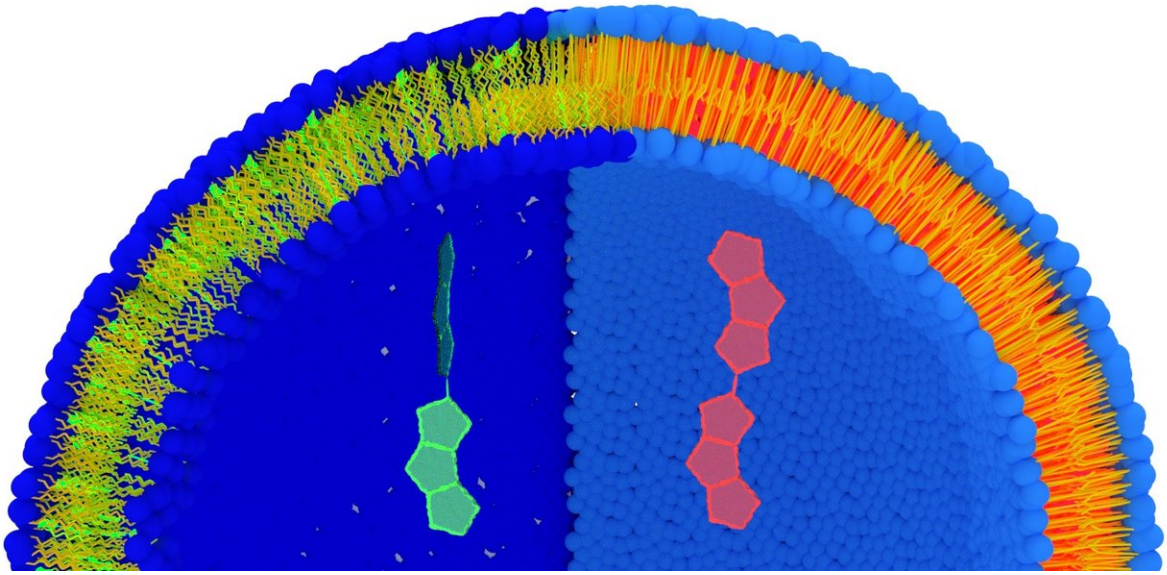

Artist's view of a lipid membrane marked with the FliptR molecule. The lipids are blue (hydrophilic part) and yellow (hydrophobic part). When lipids are stretched (left part) the molecule has a short life time (green), whereas when lipids are more compact, its life time is long (red). Both conformations are shown in large scale at the centre of the image. Credit: © UNIGE

The volume of cells can vary dramatically. Similarly to an inflating balloon, the volume increase of growing cells pushes on the plasma

membrane—the lipid envelope that surrounds the cell. This "turgor" pressure increases the tension of the membrane, which, if left uncorrected, will ultimately cause the cell to burst. To prevent this from happening, cells have evolved mechanisms to monitor the tension of their plasma membrane. When tension is too high, cells respond by increasing the amount of lipid in the membrane. Conversely, when tension is too low, cells remove lipid from the membrane to "tighten" it. How cells manage to sense tension and trigger the appropriate biological response has remained a mystery. It has been difficult to solve due to a lack of tools to study membrane tension within living cells. To tackle this problem, researchers from the University of Geneva (UNIGE) and the National Centre for Competence in Research Chemical Biology (NCCR) have collaborated to create a fluorescent molecule to measure the tension of the plasma membrane of live cells. Using this new tool, they were then able to discover how cells adapt their surface to their volume. These results, published in *Nature Chemistry* and *Nature Cell Biology*, pave the way for many applications, including in the detection of cancer cells that typically display aberrantly high membrane tension.

When the volume of a cell increases, the tension exerted on its membrane increases, causing activation of TORC2—a complex of proteins that creates warning signals within the cell. "The cell membrane consists of lipids organized into a semi-permeable bilayer," explains Aurélien Roux, professor in the Department of Biochemistry of the Faculty of Sciences of UNIGE and member of the NCCR. "This surface is fluid, allowing great adaptability of the membrane to changes in the shape and the volume of the cell. Like any surface, it can be stretched and the space between lipids then increases. When this space becomes too big and the membrane is in danger of breaking, a protein, named Slm1, activates TORC2 to produce signals that push the cell to produce new lipids and in turn reduce the tension of the cell membrane." But how could the researchers measure the tension needed to trigger this process?

To evaluate the tension of the cell membrane, it is necessary to be able to measure the space between the lipids that constitute this membrane. Stefan Matile, professor in the Department of Organic Chemistry of the Faculty of Sciences of UNIGE and member of the NCCR, has created a "probe molecule" called FliptR (Fluorescent Lipid Tension Reporter), which integrates spontaneously between the lipids of the plasma membrane. "We have developed a fluorescent molecule with two small "fins" that define a certain angle between them, he explains. This angle varies according to the pressure exerted on FliptR, which changes its fluorescence." Taking advantage of this difference in the fluorescence properties of the molecule, the group of Professor Roux was able to measure the space between the lipids and therefore the tension of a membrane.

FliptR is a valuable new tool for measuring the tension of the plasma membrane in living cells. "We know that cancer cells have higher membrane tension than normal cells. We hope that this fluorescent molecule will one day help to detect them more easily," says Stefan Matile.

And when it comes to reducing the tension of the cell?

When the tension of the plasma membrane increases, TORC2 is activated and this triggers the production of lipids to lower tension back to basal values. But what happens when the tension of the membrane is too low and has to be increased? "We initially thought that it was happening through the same mechanism running in reverse, but the story turned out to be much more interesting," says Robbie Loewith, professor in the Department of Molecular Biology of the UNIGE Faculty of Science and also a member of the NCCR. Indeed, initial research showed that the TORC2 activator Slm1—involved in sensing high membrane tension—surprisingly plays no role in the response to too little tension. "On the other hand, we observed that a particular lipid

present in the plasma membrane, called PIP2, is the sensor for low membrane tension."

When membrane tension decreases, PIP2, previously mixed with other lipids, self-segregates to form PIP2 "islands" in a sea of remaining lipids in the membrane, in a process not unlike the spontaneous separation (rising) of cream in fresh milk. As one of the proteins of TORC2 binds PIP2, TORC2 also redistributes to these PIP2 islets. Once engulfed by these islets, TORC2 becomes inactivated. "The lipids of the cell membrane are naturally degraded, and TORC2 activity is necessary to replace them" Robbie Loewith explains. But when TORC2 is inhibited within the PIP2 islets, the degraded lipids are no longer replaced, resulting in an increase in the tension of the plasma membrane. If this recalibration process is blocked, cells cannot adjust the tension of their plasma membrane and die.

A chemical measuring tool to help research in biology

Thanks to the tension measurement technique developed by Stefan Matile and Aurélien Roux, the teams of Professors Roux and Loewith could carry out their experiments on yeasts and measure the tension variations of the plasma membrane. Membrane tension is a very important parameter to control in all cellular processes in which membranes are involved, such as motility, endocytosis (the process through which the cell feeds itself), or cell division, and especially in the case of cancer development. The scientists are now focusing on checking whether the mechanism observed in yeast is the same in human cells, with the long-term idea to develop drugs capable of regulating TORC2, or even of preventing the development of certain cancers.

More information: Adai Colom et al, A fluorescent membrane tension probe, *Nature Chemistry* (2018). DOI: 10.1038/s41557-018-0127-3

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