SENSORS AND PROBES

Dynamic measurement of membrane charges

A FRET sensor enables quantitative characterization of electrostatic potential of membranes in living cells.

Electrostatic interactions between proteins and the inner leaflet of the plasma membrane are responsible for numerous cellular processes, including endocytosis and exocytosis as well as many cell signaling events. However, tools for monitoring membrane charges at the interface between the membrane and cytoplasm have been challenging to use for quantitative measurements.

Katharina Gaus and Yaunqing Ma, a graduate student in Gaus' laboratory at the University of New South Wales, are interested in the role of membrane charge in the formation of membrane domains, especially in T cell signaling. Gaus calls the lack of tools for such studies "a hurdle in our own research." Because of this, the researchers developed the membrane charge sensor (MCS), a genetically encoded Förster resonance energy transfer (FRET)-based probe for studying changes in membrane electrostatic potential.

The MCS is composed of two fluorescent proteins (a FRET pair) sandwiched between two distinct protein sequences that anchor the FRET pair to the inner leaflet of the plasma membrane. The first of these two sequences permanently attaches the sensor to the membrane, while the second is only attached to the membrane when the membrane potential is highly negative. In this configuration, a high FRET signal indicates negative membrane potential, while decreases in charge lead to decreases in signal. A benefit of this design is that the response is reversible.

After coming up with the design, Gaus noted that optimizations had to be made so that the sensor would have a biologically relevant dynamic range while still being sensitive to small changes in charge. This work was done through trial and error by Ma in what Gaus calls a "*tour de force*" effort.

After carefully characterizing the sensor's performance, the team used it in living mammalian cells. They showed they could monitor changes in membrane charges that occur because Ca^{2+} enters the cells, and that charged lipids contribute directly to electrostatic potential of the membrane. In a final demonstration, they tested the hypothesis that membrane charge is critical for T cell receptor (TCR) signaling. Here, they saw large changes in membrane potential at immunological synapses, and they went on to show that electrostatic potential regulates TCR signaling. Gaus notes that future work will be devoted to using the MCS tool to better understand these processes.

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RESEARCH PAPERS

Ma, Y. *et al.* A FRET sensor enables quantitative measurements of membrane charges in live cells. *Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.3828 (2017).

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