Mapping the folding process of a single membrane protein

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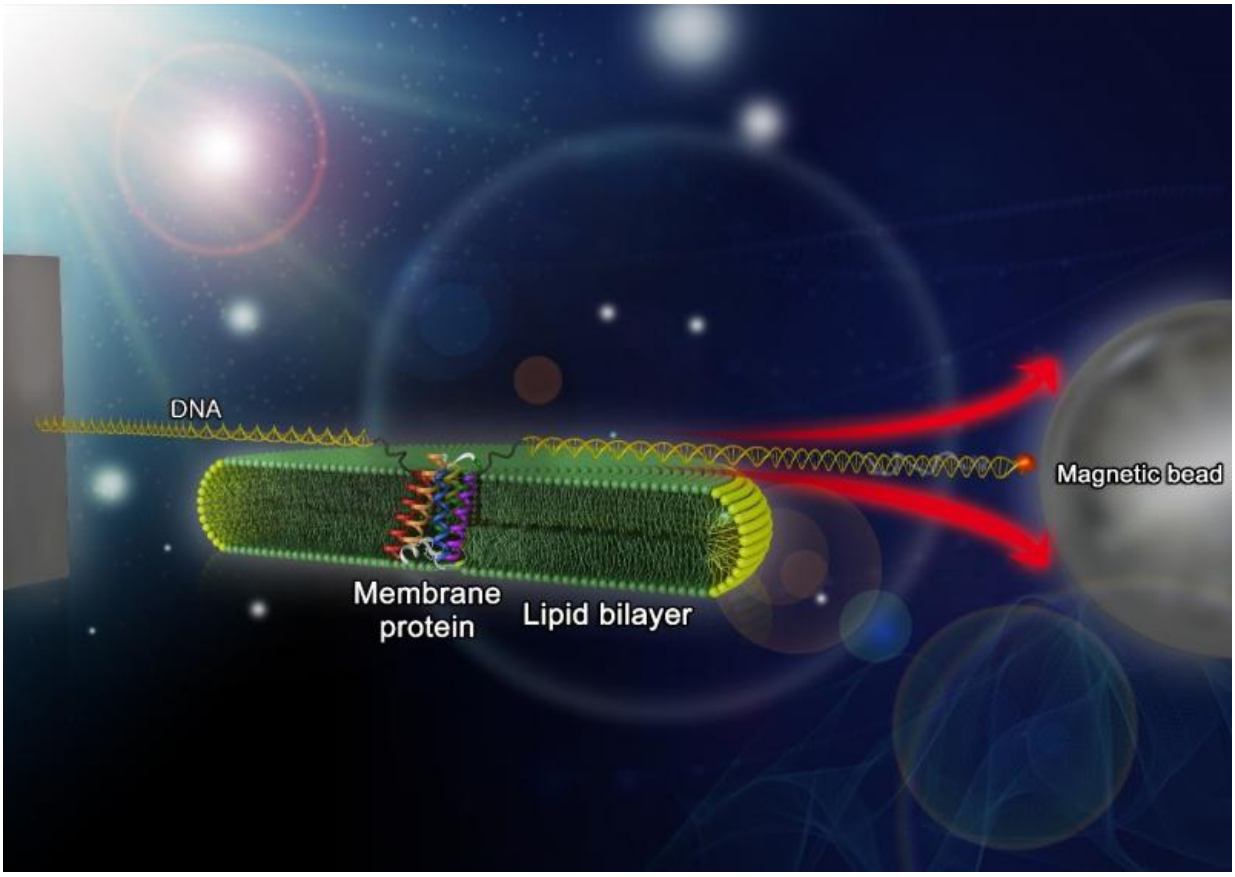

Single-molecule magnetic tweezers that induce mechanical unfolding and refolding of a single membrane protein. Since the force applied is parallel to the biological lipid membrane, the unfolding and refolding processes occur within the membrane. Credit: KAIST

Proteins are huge molecules containing hundreds to thousands of atoms that adopt a unique three dimensional structure, placing chemical groups in just the right place to catalyze reactions or build cellular structures. How all those atoms manage to find the right location - the so-called folding problem - has fascinated molecular biologists since the first structures were seen in the 1950s. Moreover, folding has important medical implications because most genetic defects cause protein misfolding.

About a third of all proteins float around in the cell membrane where they ensure the right chemicals get in the cell in the right amounts. Membrane proteins also provide key information links between the cell and its environment. Indeed, most drugs target membrane proteins. Nevertheless, the folding of membrane proteins has been particularly difficult to study and has rarely been studied in natural environments, leaving the folding process for a large fraction of the protein universe still largely cloaked in mystery.

In a recent issue of *Nature Chemical Biology*, published on October 19, 2015, a research team led by Tae-Young Yoon of the Department of Physics at the Korea Advanced Institute of Science and Technology (KAIST) and James U. Bowie of the Department of Chemistry and Biochemistry at the University of California, Los Angeles (UCLA), report a new method for manipulating the folding of membrane proteins in a membrane environment using a tool called a magnetic tweezer.

Researchers first attach long DNA handles to the ends of the protein. One handle is attached to a glass surface and the other to a magnetic bead. Using a magnet, they can essentially grab the protein and pull on it, inducing it to unfold. By playing with the bead attached to the protein, they can force the protein to unfold or allow it to refold, and watch all this happening by 3D-tracking of the magnetic bead. With this novel strategy, they were able to quantitatively map the folding energy

landscape, the folding kinetic rate, and folding intermediates of a membrane protein in a membrane environment for the first time.

"I have been dreaming about this experiment for a decade. To see it work so well is really gratifying," said Dr. Bowie.

One of the major surprises in the study was that essentially all the atoms of the protein jump into the correct structure together. The researchers expected that the protein structure would come together in a more piecemeal fashion, with different parts of the structure forming separately, but that was not the case. It is possible that nature evolved such a smooth, highly cooperative folding process to prevent partially folded forms that could get into trouble in the crowded cell membrane. On the other hand, the cooperative folding seen here might not apply to other membrane proteins.

"We need to look at more proteins. The technique developed here may allow us to do just that," said Dr. Yoon.

The single molecule mechanical manipulation technique could enable detailed folding studies of many other membrane proteins. A major barrier to the study of membrane proteins previously is that the proteins tend to stick together and get tangled up, as computer cords lying at your feet tend to do. With the tweezer technique used in this work, the protein cords are held apart from other cords so they can't get knotted up. It is hoped that the new approach will open up an important part of the protein universe to scrutiny, including many proteins that become misfolded in disease states.

More information: Mapping the energy landscape for second-stage folding of a single membrane protein, DOI: 10.1038/nchembio.1939