Researchers reveal insights into hidden world of protein folding

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The proteins upon which life depends share an attribute with paper airplanes: Unless folded properly, they just won't fly.

But researchers have been puzzled by how the long, linear proteins cranked out by the ribosome factories in a cell are folded into the shapes they must assume to perform their function. They only have known that for many of the most complex and essential proteins, the folding takes place out of sight, hidden in the inner cavity of a type of molecule called a chaperonin.

Now Stanford researchers have begun prying open the lid, literally, on the inner workings of chaperonin molecules by deducing the mechanism by which the lid operates on a barrel-shaped chaperonin called TRiC.

"Understanding how the lid opens and closes really helps us understand how everything moves inside the chaperonin," said Judith Frydman, associate professor of biology and one of two senior authors of a paper published online this week in *Nature Structural & Molecular Biology.*

"This is just the beginning, but now we can start to understand how the protein is pushed inside the cavity of the chaperonin and what this folding chamber looks like," Frydman said. Learning how a protein is manipulated inside TRiC while it is being folded is a crucial step in Frydman's larger plan.

"Our goal is to eventually exert control," she said. "If we could re-

engineer the chaperonin to either fold better misfolded proteins or alternatively to remove them from circulation, then we could prevent those proteins from being harmful to cells."

Misfolded proteins have been implicated in a number of diseases, including some cancers, as well as ailments related to aging, such as Alzheimer's and Parkinson's diseases.

"Folding is one of the key steps for the health of the cell," Frydman said.

Virtually all proteins have to be folded—some in complex configurations—in order to function properly, and many are known to require a molecule called a chaperone to fold them. Frydman estimates that perhaps 10 percent of the proteins needing chaperones must have one that, like TRiC, is part of the subset called chaperonins. Other work done in Frydman's lab has shown that proteins that have very complex folds seem to require chaperonins.

"Many of the proteins that have these complex folds are the most important ones for life," Frydman said. "The proteins that control the cell cycle, tumor suppressers and the proteins that control the shape of the cell are dependent on chaperonins to get to the folded state.

"If the chaperones don't work well, then all these proteins that have been made become toxic," she said.

TRiC, like all chaperonins, consists of a double-ringed structure that gives it a barrel shape. One ring opens to admit the raw protein into the inner recesses of the folding machine, then closes tightly while, inside the chaperonin "black box," the mysteries of molecular origami unfold—or, more correctly, fold. Upon completion of the folding, the ring at the other end opens up to push out the finished product.

"It is really like a nanomachine. It closes off, the protein is trapped inside and something—we don't understand what—happens inside this chamber, and the protein comes out folded," Frydman said. "It is a very complex mechanism."

The rings at each end of the barrel have to synchronize their actions for the sequence of events to happen correctly.

"We don't know how the rings coordinate," Frydman said. "What we have is evidence that this machine works like a two-stroke motor, so that opening one ring closes the other, and when that other ring opens, the first one is closed."

Timing is critical because if a protein does not stay in the chaperonin long enough, it may not have time to fold properly. Conversely, if it lingers too long, it may also fold incorrectly. And sometimes proteins are not made correctly by the ribosome, so they simply do not bind well to their chaperone, making proper folding impossible.

Frydman discovered TRiC in 1992. She determined that it was important for folding some of the essential proteins and had a complex structure, but was stymied in her efforts to unravel its workings because the technology needed to peer into TRiC's inner sanctum did not yet exist.

Recently, she began an interdisciplinary collaboration with Wah Chiu, a professor at Baylor College of Medicine in Houston, Texas, who is also director of the National Center for Macromolecular Imaging, located at Baylor.

Through combining biological experimentation with high-resolution imaging and computational modeling, Frydman and Chiu (the other senior author of the paper in *Nature Structural & Molecular Biology*) succeeded in uncovering how the lids at either end of the TRiC

chaperonin open and close.

"What we found is that this lid opens like the iris of a camera," Frydman said. "Previously, it was thought that the TRiC opens its lid like the flaps on a cardboard box and that the molecular machine didn't really change shape."

The motion of the lids has major implications for what happens inside the molecule.

"What has been so intriguing is that everything is connected," she said. "This is a very large machine and every part of the machine is communicating with the other parts."

Being so interconnected means that when the lids on the TRiC are twisting open and shut like the aperture on a camera, that rotation is transferred into the interior of the chaperonin. That has provided Frydman with important information on how a protein might line up inside the folding apparatus and how it begins to fold up once the lid is shut.

What they are learning has immense promise from the point of view of protein engineering and production, as well as potential for novel therapeutics, Frydman said.

"If one could understand what the environment in there looks like, what this machine does, what the cell does to fold its proteins, then we could begin to design ways to fold proteins for therapeutic purposes," she said.

Chiu and Frydman are co-directors of the Center for Protein Folding Machinery, an interdisciplinary center that is part of the Nanomedicine Initiative of the National Institutes of Health. The center integrates biology, physics and computational research from groups at Stanford,

Baylor, Massachusetts Institute of Technology, University of California-Berkeley and UCSF. Researchers at the University of California-San Francisco also contributed to the published work.

Source: Stanford University