

Protein pulling -- Learning how proteins fold by pulling them apart

Rice University physicists have unveiled an innovative way of finding out how proteins get their shape based on how they unfold when pulled apart. The experimental method could be of widespread use in the field of protein folding science, which has grown dramatically in the past decade, due in part to the discovery that misfolded proteins play a key role in diseases like Alzheimer's and Parkinson's.

Rice's new findings, which were three years in the making, are available online and slated to appear in an upcoming issue of *Physical Review Letters*. The article describes a new method scientists can use to map out exactly how much free energy is required throughout the folding process.

"We believe the method can be applied to any protein," said lead author Ching-Hwa Kiang, assistant professor of physics and astronomy. "Many people are working on this problem, and when we present our work at scientific conferences it often creates a good deal of excitement."

If DNA is the blueprint for life, then proteins are the machines built from those blueprints. All living cells produce proteins by stringing together strands of amino acids based on the sequences of their DNA. Proteins are created in linear chains, like strands of pearls, with each amino acid representing a bead on the strand. However, knowing the order of the amino acids in the strand gives no clue about how a protein functions. That's because every protein folds into a three-dimensional shape within about one second of being made, and it is this shape that dictates the protein's function.

By studying how much free energy it takes for a protein to fold into its final shape, scientists hope to learn more about how amino acid sequences affect protein function and how folding goes awry, as with some diseases.

At the halfway point between its folded and unfolded state, a protein is like a rollercoaster balanced at the crest of the highest hill on the track. Like the rollercoaster, the protein requires a certain amount of energy to make it over the hill and wind its course to a final resting place -- its folded state. If it lacks the energy to clear the hill, it will slide back into a partially folded or misfolded state.

Kiang and graduate student Nolan Harris's new approach to probing these energy states yields something akin to a map of the rollercoaster's path. For example, theirs is the only experimental method that can reveal the slope and height of the energy barrier that the protein must overcome.

"Other experimental methods give researchers a pretty clear picture of the energy states at the beginning and the end -- the two equilibrium states," Kiang said. "Our approach helps fill in what happens in between, when the system is between folded and unfolded."

Kiang and Harris's experiments were conducted on one piece of a protein named Titin. The Titin piece, dubbed I27, contains 89 amino acids. Harris suspended thousands of intact, folded I27s in a dilute saline solution and let the solution sit long enough for the proteins to become stuck to the bottom of the sample dish. The needle from an atomic force microscope (AFM) was repeatedly dipped into the solution. The tip of the AFM operates much like a phonograph needle. The AFM needle is on the end of a cantilever arm that bobs up and down over the sample. The tip of the AFM needle is just a few atoms wide. Bobbing down, it randomly grabbed I27s that were pulled into their string-like, unfolded shape as the needle rose.

Harris measured the force exerted on the cantilever arm each time an I27 was unfolded. To get the energy

maps, he wrote software incorporating a statistical mechanics equation called the "Jarzynski equality." The equation related the non-equilibrium energy from the unfolding events to the equilibrium profiles along the trajectory from the folded to the unfolded state. Kiang said the software, and the use of the Jarzynski equality, makes the new method unique and useful.

"Christopher Jarzynski only discovered this relationship 10 years ago," Kiang said. "It's a very powerful technique."

Source: Rice University

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