

Engineers squeeze secrets from proteins

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Proteins, one of the basic components of living things, are among the most studied molecules in biochemistry. Understanding how proteins form or "fold" from sequenced strings of amino acids has long been one of the grand challenges of biology.

A common belief holds that the more proteins are confined by their environment, the more stable - or less likely to unfold - they become. Now, as reported on the cover of the March issue of *Biophysical Journal*, a team of chemical and biological engineers from UW-Madison shows that premise to be untrue. While confinement plays an important role, other factors are also at play.

"Most research in this area looked at proteins in free solution when in fact, most proteins are confined in some way," says Juan de Pablo, a chemical and biological engineer at the University of Wisconsin-Madison. "What we demonstrate for the first time is that the stability of proteins under severe confinement, which is really the relevant way of looking at them for numerous applications, depends on their shape, their size and their interactions with the environment. It is a delicate balance between the energy available to fold the protein and entropy, or its desire to be in the unfolded state."

De Pablo's research team developed a method to precisely calculate the entropy and determine how much of a protein's stability change upon confinement to attribute to energy and how much to entropy. "This is the important part of the calculation," de Pablo adds.

Protein stability is an incredibly important property in myriad applications, de Pablo says. Consider laundry detergent. A popular ad for detergent once claimed that "protein gets out protein." The idea behind this is that engineered enzymes are at work in the wash breaking down elements of a stain.

"Once a protein is folded, you can actually unfold it or destabilize it, either by heating it up, or by adding solvents to the system, like urea for

example, that just destroy the folded structure of the protein. How resilient the protein is to these assaults is what we often call stability," de Pablo says. "Detergents like the ones you use to wash your clothes have enzymes that break the fat in stains. When you put your clothes in hot water in your washing machine, you want your detergents to withstand those high temperatures. So what people do is engineer enzymes that do not unfold when you put them in hot water. They design enzymes that are more stable than normal enzymes at high temperatures."

To better understand protein folding, de Pablo's team built computer models of proteins under different types of confinement. These models were then simulated to gain a better understanding of protein stability. Working with the UW-Madison Nanoscale Science and Engineering Center (NSEC), funded by the National Science Foundation, the researchers will continue to refine their models with the goal of confining, folding and measuring the stability of proteins under more realistic conditions.

Source: University of Wisconsin-Madison

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