# Proteins pack tighter in crowded native state

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The syrupy soup of proteins, ribosomes and membranes inside a living cell is so tightly packed it may increase the structural content of proteins by as much as 25 percent, according to new research from Rice University and the University of Houston (UH). The study is one of the first aimed at determining how the crowded environment inside a living cell affects protein structure.

"Based on accepted theories, we expected crowding to affect proteins in the unfolded state," said Rice biochemist Pernilla Wittung-Stafshede, one of the study's co-authors. "We were surprised when both experimental evidence and computer simulations showed that crowding also acts directly upon proteins in the folded state."

Living cells are crowded places. They're filled with a chemical soup of 100-300 mg per mL of large molecules, such as DNA, proteins and ribosomes. This corresponds to about 40 percent of volume occupancy.

"The consistency is very viscous," said Wittung-Stafshede. "It's something like Jell-O or the freeway at rush hour."

The study, which was co-authored by UH physicist Margaret Cheung, is available online and slated to appear in the Nov. 27 issue of the *Proceedings of the National Academies of Science*.

"Our simulations pinpointed specific places in the protein's structure where compaction was occurring and secondary structures improved," Cheung said. "This offers the first observed evidence -- both in silico

and in vitro -- for structural effects on proteins in the native state."

To find out how crowded environments affect the stability, structure and folding of proteins, Wittung-Stafshede and Rice graduate student Loren Stagg set up a series of biophysical experiments involving the protein apoflavodoxin. This is an excellent model system because it is well-characterized in dilute conditions and can be made in the lab.

Using sucrose-based polymers (inert synthetic mimics of real macromolecules), the pair created several test environments designed to mimic the gooey milieu that proteins experience inside a cell. Using spectroscopic methods, Stagg and Wittung-Stafshede then probed how the structural content as well as the thermal stability of apoflavodoxin changed as a function of added crowding agents.

At UH, Cheung and graduate student Shao-Qing Zhang used sophisticated computer simulations in a parallel set of tests. In the computer simulations, crowding was mimicked by solid spheres of the same size as the inert polymers used in the test tubes. In the end, the results from the lab and the computer on the same protein matched almost perfectly, lending weight to the final report.

The researchers found the protein's native state becomes more compact and more ordered. The secondary structure of the folded protein increased by as much as 25 percent based on circular dichroism data.

“From the simulations, it is evident that these changes occur in the ends of the helices and in the core, where the peptide chain packs better," Cheung said. Also, the unfolded state becomes more compact, as predicted by excluded volume theory. These effects on the folded and unfolded states made the native state of the protein 20 degrees Celsius more resistant to thermal perturbations.

Wittung-Stafshede said the group is following up with similar in vitro studies of several other proteins. The flavodoxin results and preliminary evidence from follow-up studies indicate that the native state of proteins -- the form they take when they are carrying out their normal functions inside living cells -- may be markedly different from the folded state that scientists most often study in the lab.

"Most lab experiments are done with purified proteins in dilute buffers," Wittung-Stafshede said. "In those conditions, the protein has more space to move around in than it would in its native environment. Our findings may have serious implications for the folding processes of proteins in cells and the structures of enzyme active sites in vivo. We are now beginning to assess the magnitude of these issues in the lab."

Proteins are the workhorses of biology, and their form and function are intertwined. Proteins are chains of amino acids strung end-to-end like beads on a necklace. The order comes from DNA blueprints, but proteins fold into a 3-D shape as soon as the chain is complete, and scientists can determine a protein's function only by studying its folded shape. It is still an open question how a long floppy chain of amino acids is programmed to adopt a unique 3-D shape in a timely manner (often seconds to minutes).

The science of protein folding has grown dramatically in the past decade, due in part to the discovery that misfolded proteins play key roles in diseases like Alzheimer's and Parkinson's.

Source: Rice University