'Rosetta Stone' protein offers new mechanism of allostery

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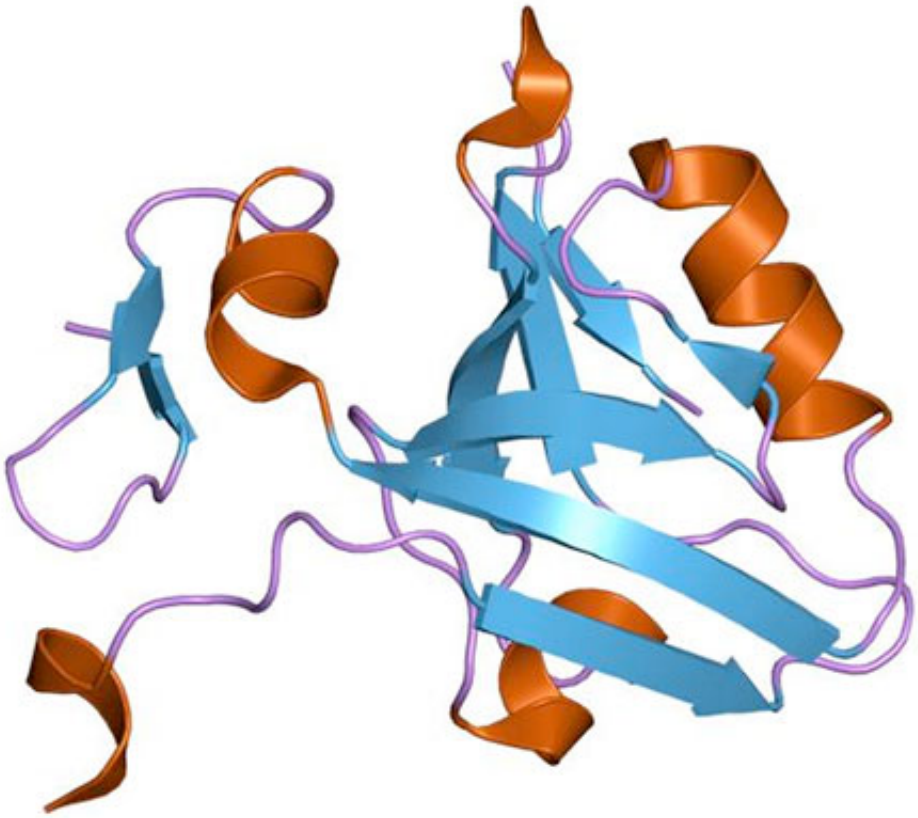

A protein cartoon of a PDZ domain from the synaptic protein PSD-95. Credit: Wikimedia Commons

For years, an unsolved problem nagged at University of Alabama at Birmingham researcher Chad Petit, Ph.D. It involved an important

biological phenomenon called allostery, a fundamental method of enzyme regulation that is crucial in living cells.

In allostery, a ligand binds to one part of the enzyme, and that binding either turns on or turns off the enzyme's active site. Since the ligand-binding site and the active site are located at different parts of the enzyme, there has to be some biophysical mechanism connecting the two changes.

The first observation of what would later become known as allostery involved hemoglobin, the protein that carries oxygen in the blood. Christian Bohr, more than a century ago, found that the presence of carbon dioxide changed the binding affinity of hemoglobin for oxygen.

Petit, now a UAB assistant professor of biochemistry and molecular genetics, had worked at UNC-Chapel Hill on a protein domain from a larger protein that is important for nerve synapse function, along with then-graduate student Anthony "Tony" Law, M.D., Ph.D. Their interest was in understanding how energy could be propagated through a protein in the absence of any discernable change in structure.

In a pioneering 2009 *PNAS* paper, Petit found that removing a small portion of the protein domain—an alpha helical string of amino acids—caused a 25-fold decrease in binding. This deletion was intended to mimic phosphorylation of the PDZ3 domain. The mechanism determined to drive this decrease appeared to be global changes in the motions of side-chains without any apparent change in the structure of the PDZ3 protein domain. It was one of the first clear-cut cases of what would be termed "dynamic allostery."

But there was one paradox, an unanswered question that gnawed at Petit even after he had moved to studying an influenza protein at UAB. That alpha helix-deletion protein was 10 percent smaller than the native

PDZ3, so it should have tumbled faster than the native protein. But biophysical experiments showed that it had an almost identical tumbling rate compared with native PDZ3.

Over the years, Petit and Law, who is now a surgical resident in otolaryngology at the University of Washington School of Medicine, kept returning to this puzzle. Finally they hatched a heretical idea—the unexpected tumbling rate must be due to an increased volume of the smaller protein.

"So, we started planning experiments," Petit said. "We started with one observation, we made a hypothesis, and then we spent years testing that hypothesis."

Every corner we looked into supported that hypothesis," Petit said. "This was pure science—it's the purest science I've ever done."

Using a grab bag of biophysical experiments with unwieldy names like HSQC spectra, NOESY analysis, differential scanning calorimetry isotherms, small-angle X-ray scattering and spin relaxation, as well as experiments in the presence of solvents that act as stabilizing or destabilizing osmolytes, Petit, Law and their research colleagues came to the following conclusion—the alpha helix-deletion protein had indeed expanded in size, and it actually was larger in volume than the native PDZ3 protein. Further, this relaxed size occurred without any discernable change in protein structure.

The bigger-volume deletion protein showed all the normal characteristics of the more tightly packed native protein, and it could be experimentally compressed through temperature changes or stabilizing osmolytes.

As Petit, Law and their colleagues probed the literature, they saw that people had seen clues of such a novel allosteric mechanism as many as

35 years ago, and various papers gave a general sense that it may be occurring in other proteins or enzymes.

This finding—at least in the realms of biophysics and chemistry—was a pretty big splash.

Their paper was accepted by the *Journal of the American Chemical Society*, which has an impact factor of 13, and they were asked to do the cover illustration. The paper was featured in the journal's JAC Spotlight, and it was also selected by the Faculty of 1000, a group of 8,000 senior scientists who recommend the most important research articles in biology and medicine.

As Petit and colleagues wrote in their paper, "The unexpected observation that function can be derived from expanded, low-density protein states has broad implications for our understanding of allostery and suggests that the general concept of the native state be expanded to allow for more variable physical dimensions with looser packing."

"It is the best paper I've done," Petit said. "For whatever reason, this deletion protein allowed us to study this mechanism. Tony calls it our Rosetta Stone."

More information: Anthony B. Law et al. Native State Volume Fluctuations in Proteins as a Mechanism for Dynamic Allostery, *Journal of the American Chemical Society* (2017). DOI: 10.1021/jacs.6b12058

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