Revealing the workings of 'Mother Nature's blowtorch'

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Using atom-level imaging techniques, University of Michigan researchers have revealed important structural details of an enzyme system known as "Mother Nature's blowtorch" for its role in helping the body efficiently break down many drugs and toxins.

The research has been detailed in a series of papers, the most recent published online this month in the journal *BBA Biomembranes*.

The system involves two proteins that work cooperatively. The first, cytochrome P450, does the actual work, but only when it gets a boost from the second protein, cytochrome b5. To complicate matters, the two proteins can interact only when both are bound to a cell membrane. That makes it difficult to use traditional techniques to discern the structural details that are crucial to the interaction, said Ayyalusamy Ramamoorthy, who leads the research group.

For instance, X-ray crystallography, often used to determine protein structures, requires separating the molecules from their membrane environment. Because part of cytochrome b5 sticks to the membrane, such separations involve breaking the molecule at the sticking point, which happens to be the part that controls its interaction with cytochrome P450. So while crystallography can offer some information on structure, it can't provide insights into exactly what goes on between P450 and b5 during their cozy, membrane-bound encounters, Ramamoorthy said.

However, the technique his lab uses—solid state NMR spectroscopy—can produce detailed images of proteins in the membrane environment, not only revealing molecular structure but also showing how a particular protein nestles into the membrane. Cytochrome b5 presented a challenge even to that versatile method, though, because the molecule has three parts that all behave differently: the rigid, sticky portion that buries into the cell membrane, a highly mobile, water-soluble portion, and a less mobile "linker" that connects the other two parts.

But by tweaking their technique, the researchers were able to get high-resolution images of all three portions.

"The challenge was something like having a room full of people and trying to get good photos of every one of them," said Ramamoorthy, an associate professor of chemistry and Biophysics. "With one picture, you probably can't do it. But if you say, 'Everyone over age 50 stand up,' and you take one picture, and then you ask for another age group and take another picture, and so on, you have a better chance."

By spinning their samples (or aligning the molecules in the magnetic field), the researchers were able to differentiate parts of the molecule based not on age group, as in the photo analogy, but by mobility. "With the techniques we designed, we were able to observe the rigid portion separately from the highly mobile and less mobile portions," Ramamoorthy said.

In the first part of the work, published in the Journal of the American Chemical Society in May, the researchers described the membrane-spanning segment of cytochrome b5, revealing for the first time its helical shape and the way it tilts in relation to the membrane. In the new work published in BBA Biomembranes, they determined that once both molecules are bound in the membrane, cytochrome b5 modulates the

motion and the structure of cytochrome P450. More work is in progress to determine the detailed high-resolution structures of these two proteins.

Ramamoorthy's team also is studying other membrane-associated proteins, a group that includes many biologically important molecules.

"These proteins are involved in all major diseases, everywhere in the body, and are therefore primary targets for pharmaceutical applications," Ramamoorthy said. "In my opinion, solving the structures of membrane proteins should be the highest priority for structural biologists in the coming years."

Ramamoorthy collaborated on the most recent work with Lucy Waskell, a professor of anesthesiology and a physician at the Department of Veterans Affairs Medical Center.

Source: University of Michigan