Advance toward nanotechy approach to protein engineering reported

June 9 2006

UCLA physicists report a significant step toward a new approach to protein engineering in the June 8 online edition, and in the July print issue, of the *Journal of the American Chemical Society*.

"We are learning to control proteins in a new way," said Giovanni Zocchi, UCLA associate professor of physics and co-author of the study. Zocchi said the new approach could lead ultimately to "smart medicines that can be controlled" and could have reduced side effects. Mimicking one essential cellular control mechanism, Zocchi's laboratory has completed an important preliminary step.

Zocchi and UCLA physics graduate student Brian Choi report one representative example where the chemical mechanism by which the cell controls the function of its proteins can be effectively replaced, in vitro, by mechanical control. Specifically, they show how an enzyme complex called Protein Kinase A (PKA) -- which plays a fundamental role in the cell's signaling and metabolic pathways, and is controlled in the cell by a ubiquitous messenger molecule called cyclic AMP -- can instead be controlled mechanically by a nanodevice that the researchers attached to the enzyme complex. The nanodevice is essentially a molecular spring made of DNA.

"Molecular biologists have been trained for 50 years to think that because the sequence of amino acids determines a protein's structure and the structure determines its function, if you want to change the structure, the way to do so is to change the sequence of amino acids. While that

approach is correct, it is not the only way. We are introducing the notion that you can keep the sequence but change the structure with mechanical forces.

"This research has many ramifications, and may lead to a better fundamental understanding, as well as new directions for biotechnology and perhaps new approaches to medical treatments."

PKA, a complex of four protein molecules, contains two regulatory subunits and two catalytic subunits. Zocchi and Choi mechanically activated PKA by placing a controlled mechanical stress on two specific points in the regulatory subunit, which causes that subunit to fall off from the catalytic subunit, activating the enzyme.

In order to obtain the desired effect, the mechanical tension is applied at specific locations in the regulatory subunit, Choi said. Knowing those locations requires a detailed understanding of the structure of the enzyme.

The research was federally funded by the National Science Foundation.

Proteins, the molecular machines that perform all tasks in the living cell, are switched on and off in living cells by a mechanism called allosteric control; proteins are regulated by other molecules that bind to their surface, inducing a change of conformation, or distortion in the shape, of the protein, making the protein either active or inactive, Zocchi explained.

Cyclic AMP (cAMP) binds to PKA's regulatory subunit and induces a change of conformation that leads to the catalytic subunit's detaching from the regulatory subunit; this separation of the two subunits is how the enzyme complex is turned on in the cell, Zocchi said.

"We can activate the enzyme mechanically, while leaving intact the natural activation mechanism by cAMP," said Zocchi, a member of the California NanoSystems Institute. "We believe this approach to protein control can be applied to virtually any protein or protein complex."

Zocchi's group first demonstrated mechanical control of protein conformation last year, when the physicists attached a controllable molecular spring, made of a short piece of DNA, to a protein and used it to inhibit its function. In the new research, the group succeeded in activating the enzyme PKA through the same principle, by using the molecular spring to induce the change in conformation that, in the cell, is induced by the natural activator of PKA (the signaling molecule cAMP).

Zocchi's group can mimic with mechanical tension the natural allosteric mechanism by which PKA is regulated by cAMP. PKA is significantly more complex than the protein that Zocchi's group used last year.

What are Zocchi's future research plans?

"I want to see whether we can make molecules which kill a cell based on the genetic signature of the cell," Zocchi said. "Cancer cells would be an obvious application. This will however require many further steps. So far, we have only worked in vitro. The exciting part is, from the outside, cancer cells can look like normal cells, but inside they carry a genetic mark.

"In the future, perhaps we can control more complicated molecular machines such as ribosomes. Many antibiotics work by blocking the ribosome of bacteria."

Source: University of California - Los Angeles