New tag could enable more detailed structural studies of mammalian proteins

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(PhysOrg.com) -- To say our genes are resourceful is a gross understatement. Through ingenious combinations of a paltry 20 amino acids, the basic building blocks of life, genes engineer all of the tissues and organs that are the marvel of our working bodies. Now scientists are adding to the parsimonious genetic repertoire to good effect: With careful targeting using genetic engineering, so-called unnatural amino acids can effectively tag proteins that scientists want to study, because, like a lighthouse beacon in a soupy fog, they stand out from the ones the body already produces.

In work published last month in Nature Chemical Biology, new research at The Rockefeller University reveals a method that could theoretically be adapted to place a fluorescent probe at any position in any protein in a mammalian cell. The new technology could enable single-molecule fluorescent studies in live cells, says Thomas P. Sakmar, head of the Laboratory of Molecular Biology and Biochemistry. “It’s a new tool to study membrane protein dynamics that should be of general use. We’re building technologies to move the science forward.”

Sakmar, research associate Thomas Huber and postdoctoral associate Shixin Ye, working with a colleague in Germany, Reiner Vogel, combined a variety of genetic engineering techniques to introduce an amino acid, azidoF, a relative of phenylalanine, into three points on rhodopsin, the light-sensitive cell receptor that is crucial to vision. The three-nitrogen-atom azido is an especially good probe for three reasons: In contrast to other tags, azido does not exist naturally in mammals,

which makes it easier to "see;" it is small enough to not interfere with a protein's normal functioning; and it has chemical properties that make it a good handle on which to hang other molecules, like fluorescent probes, says Huber.

Similar approaches have been successfully used in bacteria, but this is the first time it has been applied to mammalian cells with such specificity and efficiency, the scientists say. Extensive genetic screening allowed the team to target the azido probes efficiently. They then confirmed the presence of azido with fourier transform infrared (FTIR) difference spectroscopy, which measures stretching frequencies of the atoms in the amino acids that make up a protein. Because azido has a unique vibration frequency that is sensitive to its surroundings, the team was able to use the spectroscopic data to confirm structural changes rhodopsin undergoes in light versus dark.

"What you want is a probe that doesn't perturb the protein and one that can tell you something about its structure," Sakmar says. "That's what we have here, and in principle, you can put it at any position of any protein of interest in a mammalian cell, which will allow us to study all of the interesting proteins that can't be expressed in bacteria."

More information: FTIR analysis of GPCR activation using azido probes, Shixin Ye, Thomas Huber, Reiner Vogel and Thomas P. Sakmar, *Nature Chemical Biology* online: April 26, 2009)

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