Scientists develop fluorescent proteins for live cell imaging, biosensor design

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Scientists at Carnegie Mellon University's Molecular Biosensor and Imaging Center have developed new "fluorogen activating proteins" (FAPs) that will become a key component of novel molecular biosensor technology being created at Carnegie Mellon. The FAPs, which can be used to monitor biological activities of individual proteins and other biomolecules within living cells in real time, are described in the February issue of *Nature Biotechnology*.

Carnegie Mellon scientists designed the FAPs to emit fluorescent light only when bound to a fluorogen, an otherwise non-fluorescent dye added by the scientists. This feature will allow biologists to track proteins on the cell surface and within living cells in very simple and direct ways, eliminating cumbersome experimental steps.

Scientists say the fluorogen activating proteins are especially useful for developing molecular biosensors, because FAPs allow researchers to not only see where the target protein is within the space of the cell, but also to see color changes when it becomes fluorescent. Color changes may reflect changes in the local environment of the protein, and allow quantitative sensing in real time of the biological activity of proteins and biomolecules that are in close proximity to each other.

Biologists often have a difficult time locating a target biomolecule inside living cells using other dye technologies because of background light given off by any unbound dye molecules. This background light obscures the biomolecule's glow and therefore must be removed to successfully

carry out the experiment.

The new FAP technology gives off light only when and precisely where the target biomolecule is present, enabling scientists to activate the fluorescence when needed to see exactly where in the cell the biomolecule is located. Scientists also can design fluorogens that can enter the cell and fluorogens that can't. When used with fluorogens that are excluded from the cell, the FAP technology provides an exceptionally selective biosensor for proteins at the outside of the cell surface.

The FAP is a specialized single chain antibody (scFv), a recombinant fragment of full-size antibody proteins that the human immune system uses to identify intruders like bacteria or viruses. The Carnegie Mellon scientists screened billions of scFvs to look for those that bound specifically to either of two fluorogens, malachite green and thiazole orange. The team found several scFvs that, when bound to the fluorogen, emitted bright fluorescent signals. They termed these scFvs "fluorogen activating proteins."

"These FAPs are the essential first step in developing molecular biosensors that will monitor dynamic changes occurring within cells," said Alan Waggoner, professor of biological sciences and director of the Molecular Biosensor and Imaging Center (MBIC). "The ultimate goal is to put molecular biosensors based on FAP technology inside cells, but this current work is immediately useful. We have used the FAPs in conjunction with several fluorogens to visualize proteins at the cell surface and are now using the technology to image proteins inside cells."

The new FAPs are an extension of the genetic approach made popular by the advent of fluorescent proteins, such as green fluorescent protein (GFP), more than a decade ago. GFPs, once expressed in cells, are always aglow when visualized by scientists using special light sources

and microscopes. The Carnegie Mellon team has taken GFP technology one step further — with the novel FAPs and associated fluorogens, they can control fluorescence in space and time.

“The beauty of our system is that we can make FAPs with genetic variations so that we can co-express distinct FAPs within a cell. We can also make synthetic variations of the fluorogen that have different fluorescent and binding properties. Together, these modifications will allows us to image multiple colors inside cells, enabling us to dynamically monitor several proteins and follow complex cellular functions,” said Chris Szent-Gyorgyi, a research scientist at the MBIC who spearheaded the isolation of the FAPs.

Source: Carnegie Mellon University