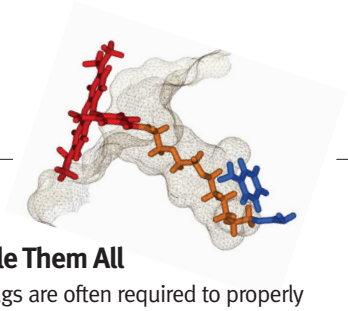
In this ISSUE

acs chemical biology

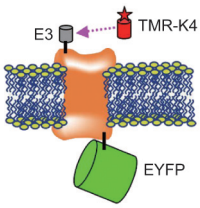


One Tag to Rule Them All

Multiple fusion tags are often required to properly study the functions of proteins. Because creating different tagged versions of proteins requires multiple cloning steps, this can become time-consuming. A more efficient method might come from a tagging system that allows linkage of different functional groups to a single genetic fusion. Los *et al.* (p 373) describe just such a system—a novel protein tag that allows labeling with different functional groups in disparate environmental milieu.

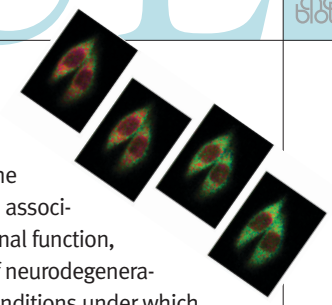
The authors started with a bacterial haloalkane dehalogenase that normally removes halides by nucleophilic displacement. Through site-directed mutagenesis, they obtain a variant that rapidly and nearly irreversibly binds chloroalkanes. Attaching different functional reporters to the reactive chloroalkane linker enables a wide array of labeling studies to be performed. To highlight the utility of this technology, the authors demonstrate fluorescent labeling and cellular localization, as well as affinity-based capture of target proteins. Synthetic approaches should allow the creation of other ligands that increase the repertoire of cellular events that can be examined with this tagging system.

Small Is Beautiful



Labeling studies that rely on the attachment of a large fusion tag to a protein of interest often suffer from technical difficulties because the tags may cause the protein being studied to misfold and aggregate. Such difficulties can be alleviated with the use of a small genetically encoded tag. Yano *et al.* (p 341) describe the design and application of a protein labeling technique that requires only a small 21-amino-acid tag.

This new method employs two helical peptides that bind to form strong heterodimers without the requirement for metal ions or additional reagents. The authors show that it is possible to specifically label surface-exposed proteins using a short peptide probe to tag the protein and another that is labeled with a fluorophore. Because the probes are not cell-permeable or toxic, they should be useful in visualizing membrane receptors.



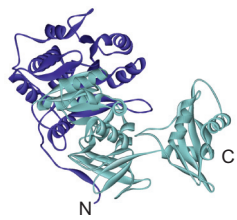
Hitting a Raw Nerve

Brain-derived neurotrophic factor (BDNF) is involved in the growth and maintenance of neurons. This polypeptide is associated with both rapid and long-term modulation of neuronal function, and a deficiency of BDNF is associated with a number of neurodegenerative diseases. However, not much is known about the conditions under which BDNF is secreted by neurons. Nakajima *et al.* (p 352) describe a cell-based assay designed to visualize and quantitate the secretion of BDNF from living neurons.

In this system, binding of BDNF secreted from neurons causes the activation of a kinase domain of a receptor. The activated kinase then phosphorylates a fluorescence-based indicator that allows rapid detection of the signal. Using this system, the authors visualize BDNF secretion from cultured hippocampal neurons that had been stimulated with the neurotransmitter glutamate. This system should be useful for the visualization of BDNF under different conditions. It should also facilitate the design of new cell-based indicators for other growth factors and cytokines.

Everything Is Illuminated

Firefly luciferase is widely used as a genetic reporter because of its sensitivity and accuracy over a wide dynamic range. Structurally, the luciferase enzyme is split into two domains that are connected by a hingelike region that closes upon binding of the substrate, firefly luciferin. Luminescence occurs when the enzyme is in this closed conformation. Fan *et al.* (p 346 and Point of View p 335) configure intracellular biosensors using luciferase as a design template.

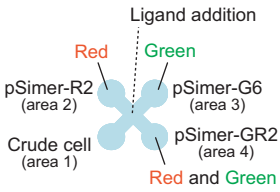


The authors describe biosensors for detecting protease activity and the small molecules rapamycin and cyclic AMP, respectively. These biosensors are designed so that specific cellular metabolites or events trigger a change in luminescence. The response dynamics of these biosensors surpass those of current FRET- or BRET-based systems. In addition, these novel strategies should be useful for designing biosensors of other covalent, noncovalent, or allosteric interactions.

Probe of Many Colors

Many small molecules such as steroids have multiple effects on signaling pathways in mammalian cells. However, it is difficult to examine these multiple effects simultaneously using conventional technologies. To elucidate the different functions of a single ligand, Kim *et al.* (p 359 and Point of View p 338) and colleagues develop a multicolor reporter system derived from split domains of the click beetle luciferase.

The authors demonstrate the generation of estrogen receptor sensor proteins using the ligand binding domain tethered to a recognition sequence. When the ligand binds, an interaction between the estrogen receptor and the tethered partner causes luminescence to occur. Using this method, the authors describe the detection of protein phosphorylation with a single bioluminescent probe. This procedure should now allow the simultaneous identification of agonism or antagonism by a ligand by observing different colors.



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