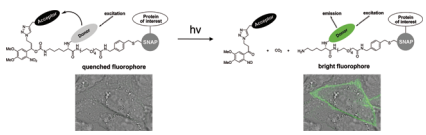
In this ISSUE

Protein Probes Light the Way

The use of fluorescence in the investigation of protein function has illuminated countless insights into molecular and cellular processes, and innovative advances in fluorescence technology along with protein engineering techniques continue to position this powerful approach at the cutting edge of research. Toward expanding the versatility of fluorescent tools available for exploring biological processes with ever-increasing temporal and spatial resolution, Maurel *et al.* (DOI: 10.1021/cb1000229) describe a general method for creating proteins labeled with photosensitive probes inside live cells.

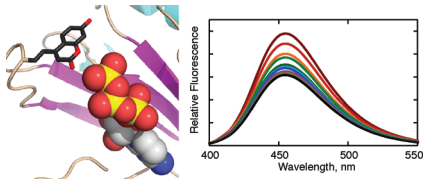
The strategy is based on the use of a probe comprised of a fluorophore connected to a fluorescent quencher *via* a photocleavable linker. Using SNAP-tag technology, which enables the generation of fusion proteins that can be chemically tagged in live cells, this probe can be chemically attached to a protein of interest, generating a labeled protein that can be rendered fluorescent upon exposure to light. The utility of the approach was demonstrated by characterizing the mobility of a G protein coupled receptor and a lipid-linked cell surface protein in live cells.



Exploring Allosteric Regulation, Unnaturally

Enzymes involved in metabolic pathways are often allosterically regulated, that is, ligand binding at a site other than the active site helps control enzyme activity. For example, the *E. coli* enzyme aspartate transcarbamoylase (ATCase), which plays a role in pyrimidine nucleotide biosynthesis, is regulated by nucleotide binding at an allosteric site. However, conflicting findings have left the mechanism of this regulation particularly puzzling. Now, using technology that enables integration of unnatural amino acids into proteins, Mendes *et al.* (DOI: 10.1021/cb9003207) report the generation of an ATCase derivative strategically designed to facilitate investigation of the mechanism of this elusive enzyme.

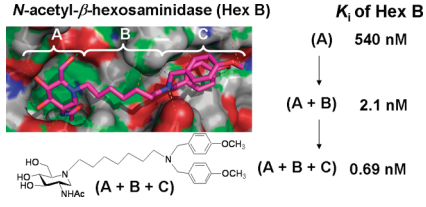
By incorporating a fluorescent amino acid into the regulatory binding site of ATCase, the effects of nucleotide binding on ATCase activity could be readily examined. The binding studies revealed that seemingly identical regulatory sites on the multimeric enzyme are functionally distinct. This functional asymmetry may enable modulation of the binding of nucleotides with differing regulatory strengths, thus offering a means to control enzymatic activity.



Putting a Hex on Osteoarthritis and Lysosomal Storage Diseases

N-Acetyl-β-hexosaminidase (Hex) catalyzes the hydrolysis of terminal glucose- and galactose-derived residues of various glycosylated biomolecules, such as glycoproteins, gangliosides, and glycosaminoglycans. Implicated in osteoarthritis and lysosomal storage diseases, Hex enzymes have emerged as intriguing drug targets. However, difficulties in finding potent inhibitors that are also selective for Hex over O-GlcNAcase, a similar enzyme that hydrolyzes glucose derivatives from O-linked glycoproteins, have hindered the drug discovery process. Ho *et al.* (DOI: 10.1021/cb100011u) now report the design, synthesis, and characterization of iminocyclitiol derivatives as potent and selective Hex inhibitors.

Their approach cleverly exploits the negatively charged active site in Hex that is conveniently absent from the active site of O-GlcNAcase. Specifically, iminocyclitiols incorporating groups strategically located to engage in additional interactions in the Hex active site were created. The compounds were shown to be effective and selective inhibitors of Hex function in human cells lines, offering an exciting entrée into development of such compounds as therapeutic agents.



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