MICROSCOPY TODAY

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The power to resolve

FLASH in Cells

Stephen W. Carmichael,' Mayo Clinic

The ability to attach fluorescent molecules onto proteins has allowed specific localization of these proteins within living cells. The traditional approach has been to purify the protein of interest then attach a fluorescent (or another useful tag) molecule *in vitro*, then somehow introduce the labeled protein into the cell. Whereas this and related techniques have been very useful, there are limitations because each of these three steps is often difficult. Wouldn't it be great if we had a "lock and key" system whereby a protein of interest could be modified to be a specific "lock" and a small taggable molecule (the "key") could diffuse into the cell and plug specifically into the lock? Albert Griffin, Stephen Adams, and Roger Tsien have developed such a system

These investigators took clever advantage of the facile and reversible covalent bond formation between arsenic-containing organic molecules and pairs of thiols. The arsenic-containing compounds are trivalent and are known to bind to specific configurations such as the paired thiol groups of proteins containing closely spaced pairs of the amino acid cystein (the "lock"). Each arsenic atom is trivalent and is known to bind to a pair of thiol groups in proteins containing adjacent cysteines. This binding is responsible for much of the toxicity of these arsenic-containing compounds. The binding and toxicity can be reversed by the introduction of antidotes such as 1,2-ethanedithiol (EDT), which contain two thiols on adjacent carbons. The strategy of Griffin *et al.* was to design a peptide domain (the "lock") with four adjacent thiols, which would cooperatively bind a particular dye molecule bearing two arsenics (the "key"). They chose an alpha-helical peptide domain of CCXXCC, where C=cysteine and X=any amino acid, to be the "lock". They made and tested 14 biarsenic-containing compounds as "keys" and found one that increased its fluorescence several thousandfold upon fitting itself into the "lock". This binding persisted in the presence of a small excess of the EDT antidote. The successful "key" contained fluorescein as the dye and was appropriately dubbed "FLASH" (FLuorescein ArSenical Helix binder).

The next step was to genetically alter a protein of interest (calmodulin, for example) so that it contained the CCXXCC motif. FLASH could be combined with the antidote EDT to form FLASH-EDT_2. FLASH-EDT_2 was diffused into living cells, then the FLASH dissociated from the EDT to specifically bind to the recombinant protein and make it fluorescent. Cells not containing the recombinant protein remained much less fluorescent. Toxicity and binding to endogenous pairs of thiols were minimized by the presence of a little excess EDT. Interestingly, a big increase in EDT concentration was able to kick the FLASH back off the protein and shut off the fluorescence, which means that this technique permits temporal control; the binding of FLASH can be turned on and off at will. Furthermore, Griffin *et al.* pointed out that molecules similar to FLASH could be designed with tags other than fluorescent molecules; one could incorporate luminescent or magnetic resonance reporters, environmentally sensitive fluorophores or indicators, or photochemically reactive moieties. These "keys" could bind to proteins containing the "lock" (CCXXCC) and confer the appropriate spectroscopic priorities.

This new system combines high affinity and speciificity, easy reversibility, easy modification of the ligand, small size of the peptide domain (CCXXCC), physiologic compatibility (due mainly to small sizes that shouldn't interfere with protein functions), plus a clear fluorescent signal (>1,000 fold increase) that binding has occurred. I predict that many investigators will be using this clever system in the future to pin down the location and function of many proteins within living cells.

1. The author gratefully acknowledges Dr. Roger Telen for reviewing this article.

2. Griffin, B.A., S.R. Adams, and R.Y. Tsien, Specific covalent labeling of recombinant protein molecules inside live cells, Science 281:269-272, 1998.

Front Page Image
First Prize - Just For Fun Micrograph Contest

At the recent Microscopy & Microanalysis '98 Conference in Atlanta, Microscopy Today held a "For Fun" contest - with micrograph entries being a composite of two or more images, one of least being microscopical in nature. With fourteen entries and hundreds of "votes", Tina (Weatherby) Carvalho from the University of Hawaii won the first (and second) prize. Her description of this composite image is:

Jumping Spider In My Lab

Jumping spiders have 8 eyes that are quite complex and can form good images. The spider image was captured on a Hitachi S-800 SEM manipulated with Photoshop, and printed on a Fuji Pictography 4000

Third prize was won by Kurt Schenk from Material Evaluation and Engineering, Inc.