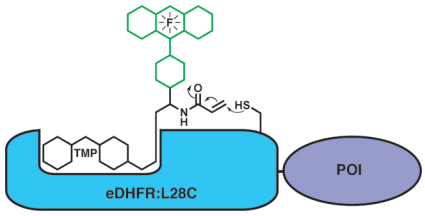
In this ISSUE

Making It Covalent

Many innovative technologies developed for exploring protein function require the use of fluorescently tagged proteins. Labeling a protein with a fluorescent small molecule is a powerful complement to the popular method of genetically fusing it to an inherently fluorescent protein. Fluorescent derivatives of trimethoprim (TMP), a small molecule ligand of the *E. coli* dihydrofolate reductase (eDHFR), have been used to noncovalently label proteins. Now, Gallagher *et al.* (DOI: 10.1021/cb900062k) engineer a proximity-induced reaction to covalently attach a fluorescent TMP tag to a protein.

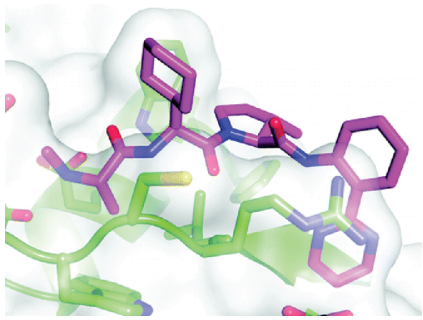
Strategic mutation of a leucine residue on eDHFR to a cysteine resulted in a variant uniquely capable of reacting with an acrylamide moiety on a fluorescent TMP molecule bound in the eDHFR active site. Genetic fusion of the DHFR variant to a protein of interest, followed by exposure to the TMP tag, resulted in a fluorescently labeled protein. This general method for covalently labeling proteins with fluorescent small molecules can be expanded to other small molecule−protein pairs, increasing the tools available for generating fluorescent proteins and paving the way for multiplexing applications.



The Path to Suicide

Strange as it may seem, cells possess a highly regulated system to commit suicide. This process, called apoptosis, is critical for maintaining proper cell and tissue function, but when it goes awry, diseases (most prominently cancer) can result. Members of the inhibitor of apoptosis (IAP) protein family, such as cellular IAPs (c-IAPs) and X-chromosome-linked IAP (XIAP), are key regulators of apoptosis, and IAP antagonists are promising anticancer agents. Ndubaku *et al.* (DOI: 10.1021/cb900083m) design and characterize IAP antagonists specific for c-IAPs, revealing valuable insight into the roles of the different IAP family members in the regulation of apoptosis.

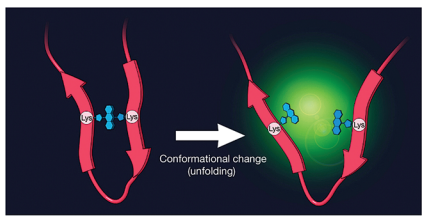
Most IAP antagonists inhibit both c-IAPs and XIAP. Using structure-based design methods and exploiting a subtle but key difference between the binding site of c-IAP and XIAP, small molecule inhibitors selective for c-IAPs were created. While the c-IAP selective inhibitors retained similar potency against the c-IAPs, they were much less efficient at inducing apoptosis than compounds that target both IAP types. The results suggest that both c-IAP and XIAP activity is needed for efficient cell death induction.



The Making of a "Smart" Probe

Use of fluorescent compounds for *in vivo* molecular imaging can facilitate the diagnosis and treatment of various diseases. However, fluorescent molecules typically have poor tissue penetration, limiting their utility for these applications. To overcome this limitation, Ogawa *et al.* (DOI: 10.1021/cb900089j) exploit an inherent property of some fluorescent molecules and create "smart" probes that fluoresce only upon binding their target.

At high concentrations, the fluorescent molecule rhodamine homodimerizes, a process that results in fluorescence quenching. Taking advantage of this phenomenon, the rhodamine derivative TAMRA was conjugated to a cancer-targeting protein. Subsequent dimerization and fluorescence quenching resulted, presumably due to crowding of the fluorophores at specific sites on the protein. However, upon the conformational change of the protein associated with binding its target, the TAMRA dimers dissociate, resulting in a fluorescence signal. A "smart" probe based on this design was created and used to image tumors in mice. Further development of this concept will facilitate the design of additional activatable "smart" probes as *in vivo* imaging agents.



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