PROTEIN BIOCHEMISTRY

FlAsHing the neighbors

Using new label-transfer chemistry, researchers study protein-protein interactions in complex environments.

The interaction of a protein with others in its vicinity is often crucial for function. But probing for such nearest protein neighbors is no straightforward task.

Thomas Kodadek and colleagues at the University of Texas Southwestern Medical Center came to this problem from studies of interactions between two large protein machines, the RNA polymerase II complex and the proteasome. Existing methods to study protein interactions involve covalent modification of the bait protein with a cross-linking reagent. This can result in disruption of interactions or function. "What we wanted to do was to get around prior covalent modification," explains Kodadek, "because then we could label a protein within a native complex."

The researchers adapted biarsenical FlAsH reagents for label transfer. They synthesized a FlAsH derivative including a biotin and a DOPA (3,4-dihydroxyphenylalanine) residue. Tagging the bait protein with a tetracysteine-containing peptide causes FlAsH to bind with high affinity to the protein of interest. Subsequent oxidation changes the DOPA moiety into a high-efficiency cross-linker to nearby nucleophilic amino acids. Thus the biotin label is transferred from the bait protein to its nearest neighbors.

Kodadek and colleagues validated their approach first by examining model proteins and then by identifying proteasomal proteins that interact with the VP16 transcriptional activator *in vitro*. Notably, only two proteins within the large proteasomal complex were labeled, suggesting that nonspecific labeling is unlikely to prove a problem.

The obvious next step is to use this methodology in cells. As Kodadek emphasizes, "there is nothing about the cellular environment that should stop the cross-linking from working inside of living cells." Although the reagent used in the present study is not cell-permeable, FlAsH conjugated to DOPA is. "So all we have to do is swap the biotin for something more cell-permeable," Kodadek says, "and that's what we're doing now."

Once this has been accomplished, researchers will have a useful tool to probe protein interactions *in situ*, within the living cell.

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RESEARCH PAPERS

Liu, B. *et al.* Label transfer chemistry for the characterization of protein-protein interactions. *J. Am. Chem. Soc.* **129**, 12348–12349 (2007).