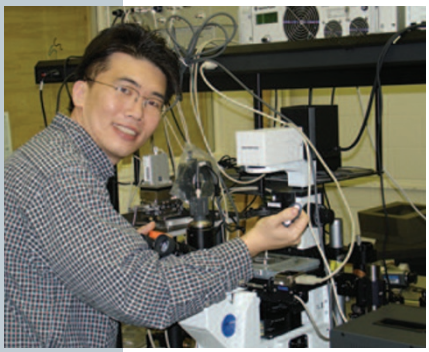
THE AUTHOR FILE

Taekjip Ha

Labeling proteins for single-molecule studies

During DNA repair and replication, various enzymes form a team of highly coordinated players. For Taekjip Ha, at the University of Illinois, Urbana-Champaign, the best way to learn the rules of the game is to watch individual molecules. "You can learn so much about the protein that you couldn't have otherwise," he says.



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But imaging individual proteins in action is easier said than done. And one of the hardest steps comes right at the beginning. To visualize the movements of single proteins, they must be labeled efficiently and precisely. "Putting the tag on the protein has been a difficult process," says Ha. "Sometimes it can be done easily; sometimes half a PhD is spent engineering a protein."

One oft-used method is attaching dye molecules to the reactive amino acid cysteine. Researchers first strip away naturally occurring cysteines within the protein of interest and then insert a cysteine exactly where the label should go. Unfortunately, the polymerases Ha's lab wanted to study had many cysteines, foiling that strategy. Other techniques, such as incorporating unnatural amino acids or inserting a fluorescent protein, were also problematic. They often produce proteins with altered function or yield too little properly labeled protein and can be difficult to implement.

During a search of the literature, Ha's postdoc Xinghua Shi thought he had found a solution, a peptide just six amino acids long with a cysteine that could be converted into a chemical form to which a wide variety of dyes would readily attach. More specifically, the amino acid sequence positions cysteine in such a way that it will be converted into the amino acid formylglycine when exposed to the appropriate enzyme inside the cells or in a test tube. Formylglycyine contains a functional group known as an aldehyde group, which is labeled by a wide variety of cyanine dyes, even if there are exposed cysteines on the protein.

Unfortunately, the conditions necessary for labeling were very harsh compared with those found in cells, so labeling proteins also denatured them. "I was very frustrated," recalls Shi. "I could see my protein labeled in the gel, but when I looked at biological function, it was gone."

When Shi tried again using milder conditions, the labeling was slow and inefficient. Even after several days, one protein was labeled with less than 40% efficiency and the other with less than 5%. "I don't know how many people can wait for a week to get a protein labeled, but I cannot," says Shi. For one thing, many proteins don't last that long.

But the kinetics of the reaction, or how labeling efficiency changed throughout the week, indicated that higher efficiencies were possible. "So we decided as biophysicists that we would put our own efforts into this and really find out whether this method actually works," says Ha. Shi set about a systematic exploration of the relevant parameters. "He worked on this by brute force," recalls Ha. "That was the most difficult part."

It turned out that using high concentrations of dye could produce reactions with 100% efficiency. But doing so introduced another concern, explains Ha: "We had to put so much dye into the reaction that we had to worry about it being specific." That launched another set of experiments, and the results were striking. "We got absolute specificity—nothing else is labeled," says Ha.

However, the first time the paper was submitted, it was rejected without review, says Ha. "A *Nature Methods* editor said that [we should resubmit] if we [could] label the protein in cell extracts without purifying or show data that can't be obtained with other methods." Single-molecule visualization experiments rely on purified proteins, so Ha's team had not considered evaluating labeling in cell extracts, he explains. "I forwarded [the email] to my postdoc. I said, 'This is a really good idea; you have to try it.'"

> "Sometimes half a PhD is spent engineering a protein."

The team did, and it worked. "You run the gel and see all sorts of proteins, but only one band lights up," says Ha, who plans to use the labeling technique to study the stoichiometry of cellular protein complexes.

Meanwhile, Ha is confident that other labs will have little trouble using the method to label their own proteins. Already, he says, a half-dozen of his collaborators are using the technique in their labs, and one has even been able to successfully insert the peptide into the middle of the protein, rather than at the N-terminus as Ha's team did. This should considerably broaden the range of proteins that can be imaged as single molecules, says Ha. "Now we can label with very small dyes, and have additional colors in addition to the fluorescent proteins."

Monya Baker

Shi, X. *et al.* Quantitative fluorescence labeling of aldehyde-tagged proteins for single-molecule imaging. *Nat. Methods* **9**, 499–503 (2012).