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Cut-And-Paste GFP

Protein Engineering: Method opens green fluorescent protein to better scrutiny, design

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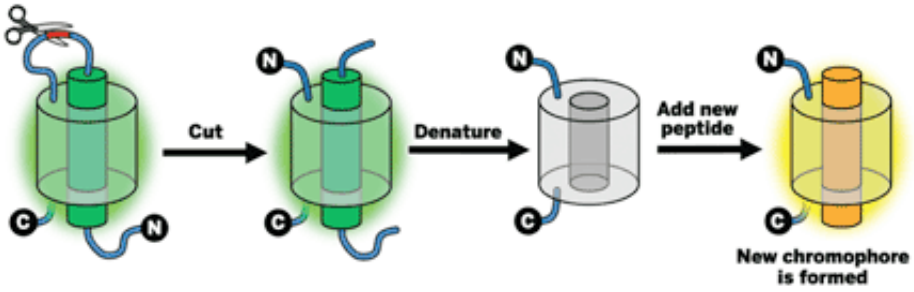

J. Am. Chem. Soc.

Replacement Parts Selected pieces of GFP can be removed by cutting a cleavage site in an inserted loop and denaturing the protein. Replacing the excised portion with a synthetic peptide restores the fluorescence.

A new in vitro method for splitting and reassembling green fluorescent protein (GFP) gives scientists synthetic control of the composition of this common fluorescent tag. The method will not only help scientists better understand the workings of GFP but also inspire the design of improved proteins for fluorescent labeling in cells.

GFP consists of β-strands that form a barrel and a chromophore-containing α-helix inside the barrel. In the new method, GFP is cut at predetermined sites, and to reassemble the protein, the parts removed by the cuts are replaced with synthetic components.

The method was inspired by so-called split GFP assays, which are widely used to detect protein interactions in vivo. In these assays, GFP is expressed as two pieces, each fused to proteins whose interaction is being probed in cells. When the proteins interact, GFP forms from the two pieces, and the assembly fluoresces. Although studying the properties of these smaller pieces of GFP in vitro would help scientists understand how this fluorescent tag works, doing so is difficult because the pieces tend to be unstable or produced at low levels.

Stanford University chemistry professor Steven G. Boxer and graduate students Kevin P. Kent and Luke M. Oltrogge avoid those problems by expressing the intact protein first and then splitting it (*J. Am. Chem. Soc.,* DOI: 10.1021/ja906303f). They engineer a loop containing a cleavage site into the protein before the portion they want to remove. They snip the protein at the cleavage site and denature the protein to pull the pieces apart. They then reassemble the protein, replacing the removed piece with a synthetic peptide.

Even before reassembly, the refolded protein with one particular β-strand removed still fluoresces, although the fluorescence differs from that of the native protein. To Boxer, the difference suggests that the refolded protein missing this β-strand still retains a rigid barrellike structure. “Otherwise, the chromophore would not fluoresce,” Boxer says.

Removing the central helix kills the fluorescence by eliminating the chromophore, but the fluorescence can be restored. “We bring back to this empty barrel a completely synthetic peptide. It has no chromophore—just the amino acids that can become the chromophore,” Boxer says. “When those two come together, that empty barrel catalyzes the reaction that

leads to the formation of the chromophore."

Boxer's group is currently using the method to dissect the chemical and physical steps of chromophore formation in GFP.

"The simple yet elegant protein-engineering approach presented here is a true success story in ongoing efforts to combine molecular biology tools with the tools of synthetic organic chemistry," says Rebekka Wachter, a GFP expert at Arizona State University. "The ability to carry out systematic, atom-by-atom chemical modifications anywhere in the protein scaffold is highly significant, as the semisynthetic approach eliminates the necessity to evolve complex enzyme systems to achieve similar goals."

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