Training GFP to fold

A method to select for proteins that are highly efficient at folding yields 'superfolder' GFP, an optimized GFP for use in protein fusions.

Fusion proteins are powerful tools for the elucidation of many biological processes. Although fusing GFP or other fluorescent proteins to a target protein is a widely used technique, it is well known that such fusions can interfere with the proper folding of the fused proteins. Not only can GFP interfere with the folding of its fusion partner, but when GFP is fused to a protein that does not fold properly, the folding of GFP is also disrupted, resulting in abnormally faint fluorescence. In fact, this phenomenon can be used to monitor protein folding and select properly folded proteins.

Waldo and colleagues at Los Alamos National Laboratory wanted to exploit this phenomenon to improve the folding ability of GFP. To do this they fused GFP to a poorly folded bait protein and screened fusion libraries created by DNA shuffling to select for brighter GFP mutants that are more efficient at folding. Waldo says, "We found that we could do that, and not only did the GFP fold better in the context of the original

protein, but also in the context of lots of other misfolded proteins. In fact, it folded no matter what was fused to it." Their improved-folding variant contained six new mutations, and they termed it superfolder GFP. As expected, the color variants of GFP could also be made from superfolder GFP, but the method is not limited to variants of GFP.

The method of producing superfolder proteins can be applied to many proteins. The researchers verified the generality of the approach with DsRed, but a similar directed evolution trajectory also should work for nonfluorescent proteins by sandwiching them between the poorly folded bait protein and a fluorescent reporter.

According to Waldo, this method results in two kinds of changes in a protein. One type of change increases the yield of folded GFP in the context of a fusion. Such mutations seem to eliminate a misfolding intermediate and should have no obvious effect on the stability of the native protein. "The surprising outcome of the study," says Waldo concerning the second kind of change, "is that we never selected for increased thermodynamic stability, and we got some

mutations that clearly mediated improved thermodynamic stability."

Waldo likens the idea of superfolders to training for a marathon. If you overtrain for the final race you will have strength in reserve. Here the training consists of evolutionary adaptation to an insult to the folding trajectory of the protein. Thus, under normal circumstances a superfolder will not look any different, but if there is any folding challenge, it will perform much better than an 'untrained' protein.

Although the performance of GFP has already been highly optimized, this work shows that there is still room for improvement. All fluorescent proteins could benefit from being made into superfolders, and although the characteristics should be carefully tested before being incorporated into sensors or fusions for fluorescence resonance energy transfer (FRET) experiments, superfolders seem certain to overcome many previously intractable problems.

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Pedelacq, J.D. *et al.* Engineering and characterization of a superfolder green fluorescent protein. *Nat. Biotechnol.* **24**, 79-88 (2006).