Differences in the folding robustness of two variants of green fluorescent protein.

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Green fluorescent protein (GFP) is widely used as a tool for studying protein trafficking, protein localization, and gene expression. The wild-type GFP folds poorly when expressed in *E. coli* (1) and even enhanced versions of GFP still exhibit folding defects. For example, the F64L+S65T variant of the commonly used cycle-3 GFP, termed "folding reporter GFP", misfolds and is only weakly fluorescent when expressed as a fusion with poorly folded proteins (2).

We have engineered a more robust version of GFP, termed “superfolder GFP”, which contains six mutations. This specific variant is useful *in vivo* for high-throughput screening of protein expression levels. Thirty-six proteins from *Mycobacterium tuberculosis** were expressed in *Escherichia coli* as fusions with either the folding reporter or superfolder GFP variants. The fluorescence of the GFP folding reporter fusions was correlated with the non-fusion solubility of the proteins expressed alone, as previously reported (2, 3). In contrast, the fluorescence of GFP superfolder was well correlated with the total whole cell expression.

Using 1.07 Å synchrotron radiation, complete, highly redundant data sets were collected for the folding reporter and superfolder GFP variants, to a resolution of 2.5 Å and 1.45 Å, respectively. Structural comparison between the two variants revealed some structural changes in the vicinity of two mutations that certainly benefit the overall stability of the β-barrel structure. Amazingly, the same mutations were found to have the most profound impact towards the increased folding robustness of GFP, according to refolding kinetics experiments.

* *Mycobacterium tuberculosis* Structural Genomics Consortium
(http://www.doe-mbi.ucla.edu/TB/DB/)

1. Crameri, Whitehorn, Tate & Stemmer (1996) *Nat. Biotech.* **14,** 315-319.
2. Waldo, Standish, Berendzen & Terwilliger (1999) *Nat. Biotech.* **17,** 691-695.
3. Pédelacq, Piltch, Liong, Berendzen, Kim, Rho, Park, Terwilliger & Waldo (2002) *Nat. Biotech.* **20,** 927-32.