

[15C03] - Advances in Protein Expression and Post-Translational Modification

Increasing demand for recombinant proteins has focused research on techniques for improving protein expression and controlling post-translational processing. We invite papers on all aspects of protein expression including novel cell systems, expression vectors, and culture conditions. Submissions dealing with post-translational processing including glycosylation, editing and secretion are also invited. Papers may deal with any cell type including bacteria, yeast, mammalian, insect and plant cell cultures.

Title: Selecting folded protein using an improved red fluorescent protein
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Abstract

Through the use of a reporter or expression gene tagged fused with the protein gene, the folding of the protein can be detected via fluorescence. Red fluorescent proteins (RFP) are easily detected via fluorimetric means and have higher emission signal-to-noise ratio compared to green fluorescent proteins. Non-aggregating red fluorescent proteins have that were recently synthesized have disadvantages such as lower quantum yield and lower fluorescence intensity. Variants with better spectral characteristics such as longer wavelength shift, higher quantum yield, and higher fluorescence intensity would be a superior folding and expression reporter. The improved RFP can be utilized as an enabling tool in expression vectors for detection of expression or folding and also in high-throughput screens such as fluorescence activated cell sorters (FACs) for purposes such as directed evolution.

Working towards the goal of engineering an improved expression reporter, we assembled the gene template for a long wavelength emitting red fluorescent protein and made mutations on the reporter gene. The mutant genes were ligated into an expression plasmid and transformed into *E. coli* cells. The transformed *E. coli* cells were induced to express the mutant fluorescent proteins and subjected to screening using FACS. Expression levels higher than 20% of cell protein were found. Longer wavelength screening was applied and the mutants having the strongest intensity were isolated and sequenced. We will report on performance enhancements as well as their relationship to positions in the protein.