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In Vivo Labeling Of A Model β-Clam Protein With A Fluorescent Amino Acid

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Abstract
Proteins can be labeled with different tags to enable their structural and functional investigations. In addition, labeling proteins at specific sites helps in studying the conformational dynamics of these molecules. A plethora of methods is available to facilitate labeling, choice of which largely depends on the requirements and the anticipated end results. In general, the various labeling methods can be classified into four different classes based on the stage at which labeling is performed, namely post translational labeling, non-ribosomal synthesis, in vitro translation and in vivo translation. Interestingly all these techniques use different unnatural amino acids for this purpose.

Protein folding is one among the many applications that requires tailoring proteins with special molecules or labels for deducing the folding pathway. Understanding the protein folding problem is a key for answering questions concerning protein behavior and thus, will provide strategies to

solve protein misfolding diseases. Protein folding is one among the unsolved problems in biology and in particular understanding the in vivo behavior of proteins in the complex cytoplasm environment with a cellular density of approximately 350 to 400 mg/ml is more critical. It is evident that there is a difference in the behavior and folding of proteins in vivo and in vitro and to deduce more insights in this aspect the protein of interest is to be labeled with a sensitive probe. The in vivo translation method offers a good method of choice for labeling the protein at a specific position and monitoring its behavior.

To study the ultimate goals of acquiring knowledge of the in vivo behavior and folding characteristics of proteins, the first step of establishing an efficient labeling technique is quintessential and as a starting step, this project aims to label a β-clam protein, cellular retinoic acid binding protein I (CRABP I) a 136 amino acid protein, with a sensitive unnatural fluorescent amino acid probe in vivo in E. coli cells.

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