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FABRICATION OF PROTEIN ARRAYS USING BIOTINYLATED AMBER CODON SUPPRESSOR tRNA.

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Protein arrays are becoming a powerful means of detecting proteins and investigating their interactions and functions. They are versatile tools for parallel screening of a large number of immobilized proteins in a time and cost effective manner. Existing arraying technologies require multiple reaction steps and modifications, such as addition of tagging molecules, to either the DNA template or the produced protein. To overcome these shortcomings, we present a method in development to create protein arrays using biotinylated amber codon suppressor tRNA, which enables simultaneous synthesis, labeling and immobilization of protein from unmodified DNA as starting molecule. In this study the incorporation of biotinylated amino acid to green fluorescent protein (GFP) at the amber stop codon (TAG) was carried out using biotinylated amber codon suppressor aminoacyl tRNA and reconstituted E. coli based coupled transcription/translation cell-free system. The commercially available amber codon suppressor tRNA is aminoacylated with biotin labeled amino acid and is engineered to recognize and enter the ribosomal A site when the ribosome is at the stop codon. By using the amber codon suppressor tRNA to label proteins we can ensure point specific labeling of full-length proteins and avoid unwanted side product. Further by removing release factors from the E. coli based coupled transcription/translation cell-free system, we can increase the labeling efficiency to full-length GFP (Figure 1). The described method offers the possibility to immobilize proteins from unknown DNA while retaining the natural state and function of proteins (Figure 2). Finally, the integration of array technology enables generation of high-density protein array for global proteome analysis.

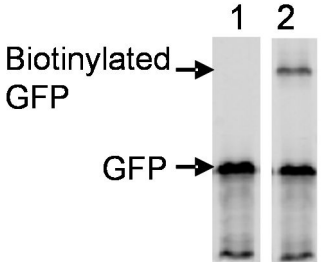

Figure 1: Comparison between protein synthesis product when biotinylated amber suppressor tRNA was (1) absent and (2) present.

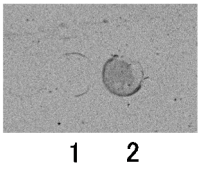

Figure 2: Comparison between immobilized protein synthesis product when biotinylated amber suppressor tRNA was (1) absent and (2) present.