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Development of Protein Microarray via Puromycin Technology

OSubhashini Raj Kumal [1], Manish Biyani [1,2], Takanori Ichiki [1,2]
1) Department of Bioengineering, School of Engineering, The University of Tokyo
2) Core Research for Evolutional Science & Technology, JST
E-mail: subhashini@bionano.t.u-tokyo.ac.jp

Protein arrays represent a class of devices that are of growing importance in the field of proteomics. These arrays enable screening of a large amount of proteins in a short time and at a lower cost. Here we present a method to fabricate protein array using biotin-conjugated puromycin to simultaneously synthesize and label proteins followed by immobilization onto streptavidin-functionalized surface based on the non covalent biotin-streptavidin interaction. This method demonstrates the fabrication of protein array based on cell-free system using unmodified DNA as a starting genetic material. As a consequence, the procedure of protein arraying has been simplified over the conventional approaches that require tedious multi-step reactions. In earlier experiments using this approach, we have observed that the efficiency of puromycin incorporation into protein was generally low and the remaining unreacted puromycin derivative competes with the puromycin-incorporated protein for immobilization onto substrate. Here we describe techniques to improve protein labeling efficiency using puromycin and thus enabling the fabrication of high density protein arrays. For this purpose, the incorporation of biotin-Cy5-labeled puromycin into DHFR protein was carried out using reconstituted *E. coli* based coupled transcription/translation cell-free system. We studied the following parameters and how variations in each of these parameters affect the incorporation efficiency: (1) concentration of puromycin derivative, (2) absence of release factors (RFs) and (3) presence of salt buffer. The yield of puromycin-labeled protein was dependent on the concentration of biotin-Cy5-CACC-puromycin used as shown in Figure 1a. We observed the lowest concentration of biotin-Cy5-labeled puromycin required to produce detectable amount of labeled full length protein with Typhoon 9400 laser scanner was 0.2μM. This concentration also produced the largest ratio of labeled full length protein to labeled truncated protein (Figure 1b). Production and immobilization of full length protein is vital when the fabricated protein array is used in applications such as protein function screening. In the absence of release factors as shown in Figure 1c, the ratio of labeled full length protein to labeled truncated protein increased from 0.1 to 3.3 at 10uM biotin-Cy5-labeled puromycin. We introduced purification procedure to remove unreacted puromycin derivative and with these improvements the amount of immobilized protein increased about 26%. We are currently working on assaying DHFR enzymatic activity on the fabricated protein array chip.

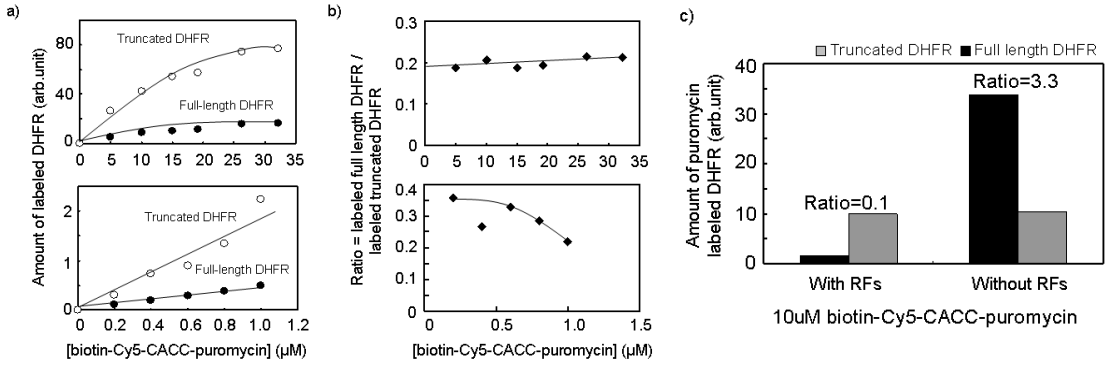

Figure 1: (a) Production of puromycin incorporated DHFR protein is dependent on the concentration of puromycin derivative. (b) The ratios of production of full-length and truncated proteins were almost unchanged at high concentration range of puromycin derivative (top panel) as compared to low concentration range of puromycin derivative (bottom panel). (c) Removal of release factors (RFs) from cell-free transcription/translation system increases labeling efficiency to full-length DHFR protein. The ratio of labeled full-length proteins with RFs to without RFs increased from 0.1 to 3.3.