Elucidating the fitness landscape of protein translation system

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The protein translation reaction[1], one of the most important regulators of cell behavior, involves a large number of components that interact with each other, and thus can be seen as a reaction system consisting of an intermolecular interaction network. It has been demonstrated experimentally that 36 proteins and ribosomes (consisting of 55 ribosomal proteins) are sufficient to carry out the reaction[2]. These minimal protein components include initiation, elongation, and release factors, aminoacyl-tRNA synthases, and enzymes involved in energy regeneration. In addition to the protein components, the protein synthesis reaction requires at least 78 non-protein components, including ATP, GTP, amino acids, and Mg ions.

We investigated the fitness landscape of the protein translation system by experimentally optimizing the system toward increased protein production yield using the PURE system[2], an *E. coli*-based *in vitro* translation system composed of only the minimum of 169 highly purified components (36 proteins + 55 ribosomal proteins + non-protein components). With this system, it is possible to not only identify the components of the system and their concentrations, but also to vary the concentrations of most of these components. A single coordinate in the 169-dimensional space, defined by the concentration of each component, gives a single value of the fitness, defined by the protein yield of that particular system. Therefore, elucidating the correlation between coordinate and the fitness will lead to not only an understanding of the protein translation system but also provide insight to allow the design of a system with improved performance over those currently available.

We investigated the additivity in the 169-dimensional fitness landscape of the protein translation system. We first chose 69 of the 169 components the concentrations of which could be manipulated experimentally. We optimized the yield of synthesized functional

GFP molecules by varying the concentrations of these 69 components. Briefly, initial concentrations of components, defined as $M_0 = (C^0_1, C^0_2, C^0_3, \ldots, C^0_i, \ldots, C^0_{69})$, where C^0_i is the concentration of component *i*, were varied by altering the concentration of a single component while keeping the others fixed and determined the optimum concentration that gave a maximum GFP fluorescence signal after 3 h of synthesis reaction. These experiments provided a list of optimum concentrations, $M_1 = (C^1_1, C^1_2, C^1_3, \ldots, C^1_{69})$. Here, we defined the GFP fluorescence intensity obtained after 3 h of protein synthesis reaction using components at concentrations M_k as R_k. We repeated the optimization procedure using M_1 as the list of initial concentrations, and then obtained the concentrations M_0, M_1, and M_2 and the fluorescence intensities R_0, R_1, and R_2 (Figure 1). While the fluorescence intensity increased from R_0 to R_1, the value was significantly reduced from R_1 to R_2 (Figure 2). These results indicate that the effects of individual components on the GFP synthesis reaction are not additive. Detailed analyses of these results with regard to the features of the fitness landscape are presented.

References

1. Nierhaus, K.H. & Wilson, D.N. Protein synthesis and ribosome structure: translating the genome. (Wiley-VCH, Weinheim; 2004).
2. Shimizu, Y. *et al.* Cell-free translation reconstituted with purified components. *Nat. Biotechnol.* **19**, 751-755 (2001).

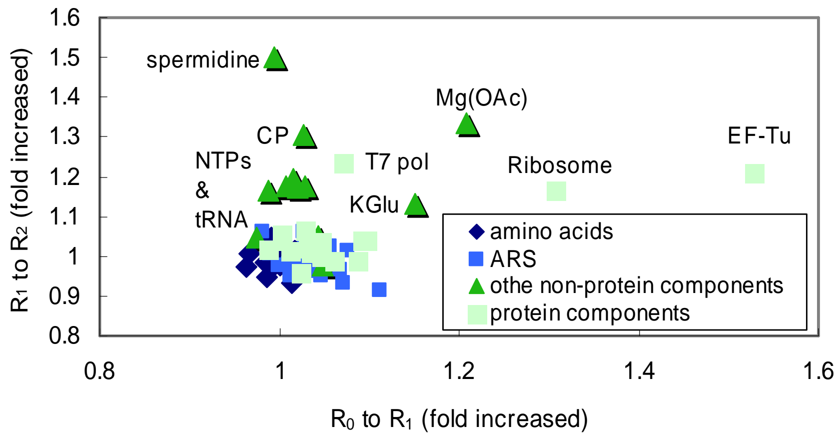

Figure 1: Optimization of the concentrations of 69 components for improved GFP synthesis reaction. The vertical axis shows the increase in GFP fluorescence intensity obtained by altering the concentration of component i from C^0_i to C^1_i, while the others remained fixed at C^0. The horizontal axis shows the increase in GFP fluorescence intensity obtained by altering the concentration of component i from C^1_i to C^2_i, while the others remained fixed at C^1. The results indicated that in the first round of optimization, altering the protein components was effective, whereas in the second round altering non-protein components was more effective in increasing the yield of functional GFP.

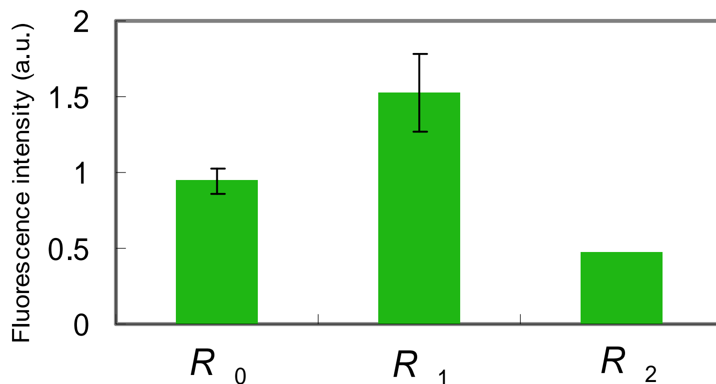

Figure 2: Increases in GFP fluorescence intensity through optimizing the concentrations of 69 components. While the fluorescence intensity increased from R_0 to R_1, the value decreased significantly from R_1 to R_2, indicating that the effects of individual components were non-additive.