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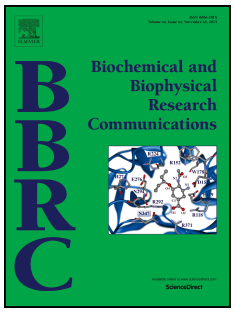

Steady-state structural fluctuation is a predictor of the necessity of pausing-mediated co-translational folding for small proteins

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Steady-state structural fluctuation is a predictor of the necessity of pausing-mediated co-translational folding for small proteins

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Abstract

Translational pausing coordinates protein synthesis and co-translational folding. It is a common factor that facilitates the correct folding of large, multi-domain proteins. For small proteins, pausing sites rarely occurs in the gene body, and the 3'-end pausing sites are only essential for the folding of a fraction of proteins. The determinant of the necessity of the pausings remains obscure. In this study, we demonstrated that the steady-state structural fluctuation is a predictor of the necessity of pausing-mediated co-translational folding for small proteins. Validated by experiments with 5 model proteins, we found that the rigid protein structures do not, while the flexible structures do need 3'-end pausings to fold correctly. Therefore, rational optimization of translational pausing can improve soluble expression of small proteins with flexible structures, but not the rigid ones. The rigidity of the structure can be quantitatively estimated *in silico* using molecular dynamic simulation. Nevertheless, we also found that the translational pausing optimization increases the fitness of the expression host, and thus benefits the recombinant protein production, independent from the soluble expression. These results shed light on the structural basis of the translational pausing and provided a practical tool for industrial protein fermentation.