Energy Analysis of Miniprotein by in Vivo Protein Crystallization

([1]*Graduate School of Life-Science and Technology, Tokyo Institute of Technology*)
○Mariko Kojima,[1] Yuki Hishikawa,[1] Satoshi Abe,[1] Tadaomi Furuta,[1] Duy Phuoc Tran,[1] Akio Kitao,[1] Takafumi Ueno[1]

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X-ray crystal structure analysis is the most essential structure determination method to gain reliable structural information of proteins at the atomic level. However, crystallization remains a bottleneck because of the difficulty in optimizing crystallization conditions to gain high-quality diffracted crystals. Protein crystal engineering to enhance crystallizability and improve crystal stability has been achieved. In recent years, immobilizing the target molecule at well-defined positions in an existing host lattice of protein crystal attracts attention as a high-versatile crystallization of the target molecule.[1] One of the challenging studies using host protein crystal is experimentally capturing the 3D structure of the target molecules in an unstable state, which is difficult to obtain in solution.

In this study, we attempted X-ray crystal structure analysis and energy analysis of miniprotein with ten amino acids, CLN025 immobilized in the loop region of polyhedra crystal (PhC) that realize high-resolution crystal structure analysis (Fig. 1a,c).[2,3] CLN025 folds to remarkably stable β-hairpin structure in solution. We designed five types of CLN025 fused PhCs with different fusion site and linker. X-ray crystal structure analysis revealed that the electron density of the full length of CLN025 was observed in one of the mutants, **ΔL1-CLN-2-PhC** in which CLN025 was inserted between Y71 and N77 (Fig. 1b). The structure indicates that the structure of CLN025 immobilized in **ΔL1-CLN-2-PhC** was an unstable helical structure that has not been observed experimentally (Fig. 1d). The energy analysis of CLN025 obtained in this measurement by the simulation is carried out at present.

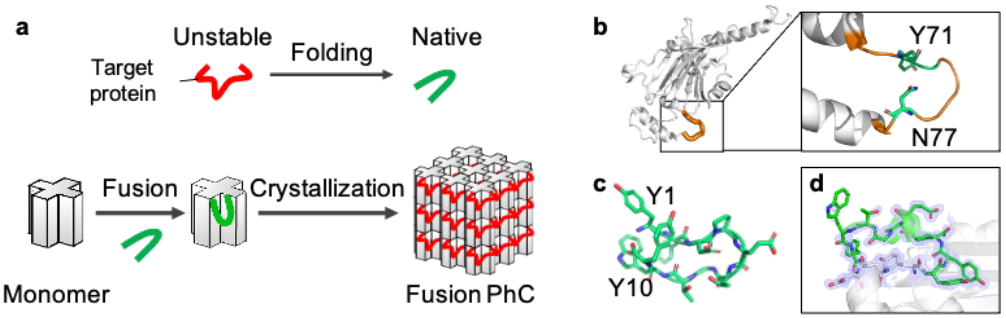

Fig. 1 a) Scheme of immobilization of unstable state target in fusion mutant PhC. b) Fusion site in loop region of **ΔL1-CLN-2-PhC**. c) Structure of CLN025 (PDB ID: 5awl). d) Structure and electron density map of CLN025 observed in **ΔL1-CLN-2-PhC**.

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