

Dynamics structural analysis of miniprotein by in-cell crystal engineering

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Protein crystals are attracted attention in the field of biomaterial science¹. The porosity of protein crystals leads to preparing the scaffold to capture the foreign molecules with various sizes, such as organic molecules, peptides, and proteins. One of the unique functions of scaffold protein crystal is to stabilize the unstable structure of target molecules by noncovalent interactions at the target-scaffold interface of crystal packing. Then, we focused on the peptide conformation controlled by the surrounding environment. In this study, we analyzed the structure of CLN025, a miniprotein consisting of 10 amino acid residues exhibiting an unstable state in a crystalline scaffold by X-ray crystallography (Fig. 1a). Polyhedra crystal (PhC) was employed as a scaffold because of the high-resolution structure. The dynamic mechanism of conformational change assisted by protein-protein interaction (PPI) was elucidated by molecular dynamics simulation.

CLN025 was inserted in the loop region of PhC, which interacts with the adjacent monomer. As a result of X-ray crystal structure analysis, the electron density of the full length of CLN025 was determined as a helix structure that had never been observed experimentally (Fig. 1b). To elucidate the pathway to fix the helix structure, targeted molecular dynamics simulation (TMD) was performed by modeling the obtained dimeric structure of PhC as the target structure. TMD indicated that salt bridge formation between CLN025 and PhC drove helix formation followed by the hydrogen bonds and aromatic interaction at both ends of CLN025. Therefore, peptide structure can be controlled by designing scaffold protein crystals. This result suggests the novel use of protein crystal as a template to observe the PPI of intrinsically disordered protein and create functional peptides.

1) M. Kojima, *et al.*, *Biomater. Sci.*, **2022**.

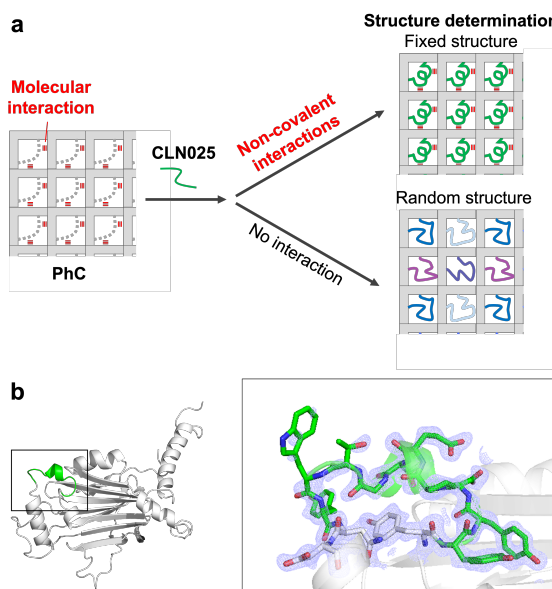


Fig. 1 a) Scheme of structure determination of unstable state CLN025 in PhC. b) The helix structure of CLN025 observed in PhC. CLN025 is colored in green.