Molecular design for immobilizing small molecules using cell-free protein crystallization

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The structure determination of organic molecules in proteins is important to understand and develop their function in biological environment. It has been reported that porous protein crystals can be applied to determine the structure of foreign organic molecules.[1] However, conventional crystallization methods require the purification and crystallization of proteins. Therefore, crystal structure determination involves a lot of time and effort. Here, we have established a procedure for immobilizing foreign molecules and their structure determination using protein crystals prepared by a cell-free protein crystallization method (Figure 1a).[2] We used Galactin-10 (Gal-10), also known Charcot-Leyden crystals (CLCs), as a template to immobilize small organic compounds such as carbohydrates or sugar-based drug molecules. [3]

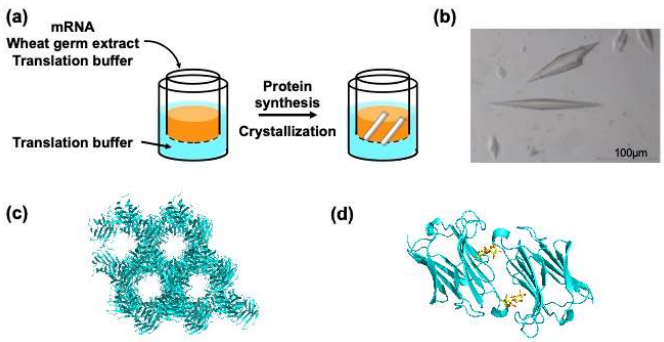

Figure 1. (a) The schematic diagram of cell-free crystallization of Gal-10, (b) Photo of Gal-10 crystal. (c) Lattice structure of Gal-10 crystal. (d) The crystal structure of WT Gal-10 homodimer complex with sucrose

Expression of Gal-10 protein by cell-free synthesis method yielded hexagonal bipyramidal crystals 200 μm long (Figure 1b). The crystals were immersed in a solution containing the target molecules to immobilize them within the crystals. Crystal structures of the Gal-10 with sucrose or lactose were determined at SPring-8 (Figure 1c, 1d). Currently, immobilization and structure analysis of other target molecules are underway. Since this technique allows crystallization and structural analysis to be performed rapidly and in small amounts in a single tube, it will become a tool for the structure determination of a wide range of target molecules, such as, drug molecules and peptides.

1)T. Matsumoto, *et al., BBRC,* **2019**, 518, 2, 402-408. 2)S. Abe, *et al., Sci. Rep.,* **2022**, 12, 16031. 3)E. K. Persson, *et al., Science* **2019**, 364, eaaw 4295.