Construction of Functional Protein Needle Materials Encapsulated in In-cell Protein Crystal

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Keywords: Protein Needle, Polyhedra crystal, In-cell encapsulation, Multiple protein interface.

In-cell protein crystals such as polyhedra (PhC) provide an advantage in the construction of solid biomaterials because they have high stability against a wide range of pH, organic solvents, and physical stresses.[1, 2] Immobilization of proteins and enzymes into in-cell crystals is a unique method for the synthesis of functional materials used as solid-state catalysts. However, such techniques based on electrostatic interactions and genetic fusions between a target protein and in-cell crystal protein are still challenging to apply to protein assemblies.

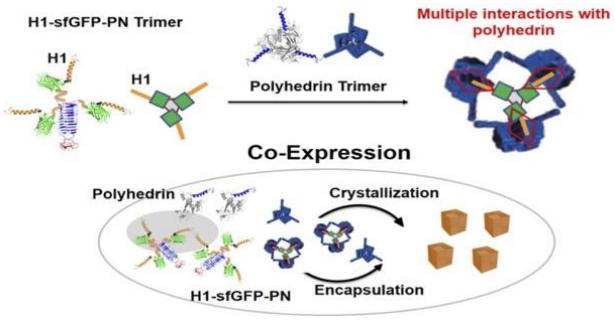

Figure 1 Construction of PN materials encapsulated in PhC using in-cell crystallization

In this work, we used the polyhedra crystal (PhC) as the scaffold to develop a new strategy to immobilize various proteins with a high encapsulation yield. Here, a protein needle (PN), a protein assembly, was used as the platform to be immobilized into the PhC. The feature of PN is to form symmetry assembly structures (trimer or trimer-dimer) even when fused with foreign proteins. Therefore, we expect the foreign protein connected to PN to be efficiently encapsulated by multiple interactions with PhC. To demonstrate this strategy, we designed GFP-PN fusion proteins. In addition, to immobilize GFP-PN into PhCs, the H1-helix derived from polyhedrin monomer (PhM) was fused to the N-terminus of GFP-PN. When H1-GFP-PN and PhM were co-expressed in the living cells, the hybrid crystals encapsulating H1-GFP-PN were constructed. This design provided a much higher encapsulation of GFP into PhC than H1-GFP. In addition, PN-based material provides cellular penetration and immunological response.[4] Therefore, this new technique opens a promising future for construction materials for biological applications such as oral vaccines or drug deliveries.

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