Enhancing biomolecule delivery in native microbial systems using cell-penetrating peptides

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Abstract

Electroporation, conjugation, and transduction are commonly used methods for introducing biomolecules into native microbes. However, these techniques often have limitations, including low transformation efficiency or restricted applicability, especially in novel or hard-to-transform microbial strains. To overcome these challenges, we investigated cell-penetrating peptides (CPPs) as an alternative approach. CPPs are short peptides capable of naturally permeating bacterial membranes, making them promising carriers for biomolecules like proteins and nucleic acids. We first optimized CPP permeation efficiency by evaluating the structure and abiotic factors that influence its effectiveness. After enhancing CPP performance, we tested CPP conjugates with peptide nucleic acids (CPP-PNAs) to regulate protein expression in several difficult-to-transform microbial strains in both prokaryotic (*Paenibacillus*, *Streptomyces*, cyanobacteria) and eukaryotic systems (haptophytes, dinoflagellates). We discovered that factors such as temperature and solution tonicity significantly affect CPP permeation. Additionally, structural modifications, such as altering the length of amino acid side chains, improved CPP efficiency. CPP-PNA conjugates successfully regulated protein translation, enabling functional analysis of key enzymes in the model strains. This approach allowed for the elucidation of protein functions and the investigation of metabolic pathways in vivo. In conclusion, CPPs offer an efficient and versatile method for delivering biomolecules into a wide range of bacterial species, providing valuable insights into bacterial metabolism and facilitating functional analysis within native microbial systems.

Keywords: Cell, penetrating peptides, biomolecule delivery, native microbes, functional analysis

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