

# Protein delivery system: Engineered bacteria as cancer killers

## Background & Innovation

Delivering proteins to the cytosol of eukaryotic cells is challenging due to the protective nature of cellular membranes. Current delivery methods, including cell-penetrating peptides, nanoparticles, and bacterial toxins, often rely on inefficient endosomal escape mechanisms. Certain bacterial secretion systems, such as the type III secretion system (T3SS), offer a promising alternative, capable of directly transporting proteins across cell membranes.

This invention leverages an avirulent strain of *Salmonella typhimurium* enhanced with T3SS to effectively deliver synthetic binding proteins, such as DARPins, into multiple cell types (Figure 1), enabling functional inhibition of key pathways like RAS signaling. This method could significantly improve intracellular protein delivery, enabling studies of complex signaling pathways like RAS and offering potential therapeutic applications in cancer and infectious diseases by directly targeting intracellular proteins.

## Efficient delivery of functional binding proteins to the cytosol

DARPins were transferred into eukaryotic cells and to confirm that DARPins remain functional post-transfer, an anti-GFP DARPIn was delivered into HeLa cells expressing Sec61-GFP, a key component of the endoplasmic reticulum translocon.

The green fluorescence of the endoplasmic reticulum aligns perfectly with the DARPins (Figure 2, GFP), demonstrating their active colocalization with GFP and suggesting that DARPins refold in the eukaryotic cytoplasm after passing through the narrow needle complex.

Furthermore, the successful targeting of nuclear proteins was achieved by delivering anti-GFP DARPins into a mixed HeLa cell line expressing both Sec61-GFP and Histone 2B-GFP (localized in the nucleus). Observing DARPins in both the nucleus and cytoplasm (Figure 2, FLAG) underlines their versatility and maintained functionality within the cell.

## FOCUS SECTORS

- Therapeutics
- Immunology
- Oncology

## PROJECT KEY WORDS

- Type III secretion system
- Protein delivery
- Intracellular protein targeting
- RAS signaling pathway

## DEVELOPMENT STATUS

- Proof of Principle

## PATENT PROCEDURE STATUS

- EP Patent application filed

## POTENTIAL FOR COOPERATION

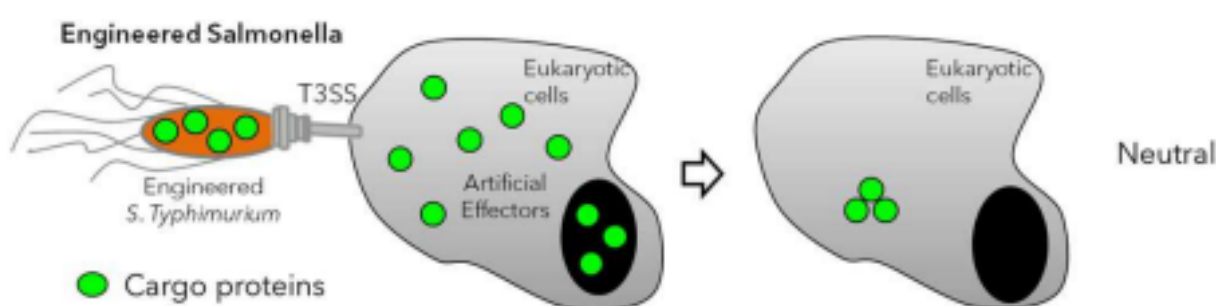
- R&D Cooperation
- Know-How exchange



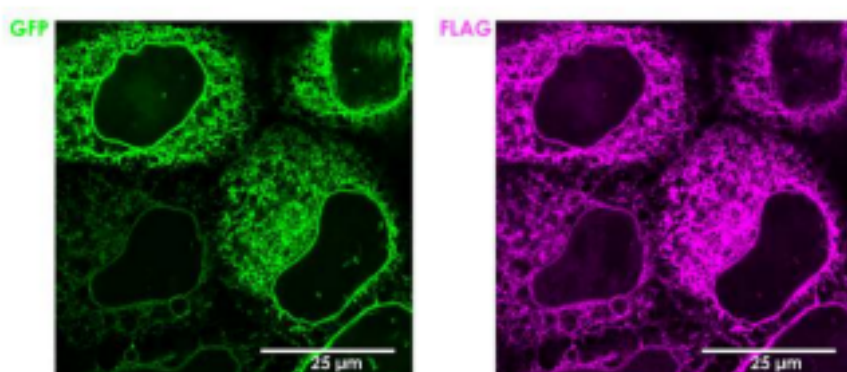
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**Figure 1:** The invention lead to an engineered delivery system for artificial effectors in eukaryotic cells using a modified *Salmonella* strain. This system allows the transfer and function of specific proteins (e.g. DARPins) within eukaryotic cells, potentially bypassing typical pathogenic entry mechanisms.



**Figure 2:** The functionality of transferred DARPins was confirmed by their colocalization, indicating that they remain active after transfer. DARPins refold in the eukaryotic cytoplasm after passing through the transfer complex.

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