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CREATE CHANGE

AVAILABLE TECHNOLOGY

Protein-coated nanoparticles for non-GM delivery of nucleic acids in plants

Key features

- Enables transient expression, gene silencing, and genome editing in intact plant tissues
- Lysozyme and NLS peptide coatings facilitate active uptake via endocytosis and membrane trafficking
- Versatile payloads (sRNA, siRNA, dsRNA, plasmid DNA, PCR fragments) and systemic translocation
- Broad compatibility across plant species and tissues

Background

The delivery of functional nucleic acids into intact plant tissues remains one of the most significant technical challenges in plant biotechnology. Conventional transformation methods such as Agrobacterium-mediated gene transfer, biolistic bombardment, and viral vectors are often limited by host specificity, low efficiency, and the potential for genomic fragmentation or epigenetic instability. These approaches typically require tissue culture and regeneration, which are labour-intensive and can introduce somaclonal variation. Moreover, the increasing demand for non-GM and chemical-free plant breeding solutions has intensified the need for alternative delivery platforms that are both biologically effective and publicly acceptable. In this context, nanoparticle-mediated delivery offers a promising route for transient gene expression, RNA interference, and genome editing without the integration of foreign DNA. However, the rigid plant cell wall presents a barrier to nanoparticle uptake, and most existing nanocarriers lack the ability to actively engage with plant endocytosis and membrane trafficking pathways.

The technology

The patented technology comprises layered double hydroxide (LDH) nanoparticles coated with proteins possessing an overall positive surface charge, most notably lysozyme and SV40-derived nuclear localization signal (NLS) peptides. These protein-coated LDH nanoparticles serve as versatile nanocarriers for a broad spectrum of nucleic acids, including mRNA, siRNA, dsRNA, plasmid DNA, and PCR-amplified DNA fragments. The LDH particles, typically 30–50 nm in diameter, are engineered to adsorb nucleic acids via electrostatic interactions and are subsequently encapsulated by the protein coating. This dual-functional design enhances colloidal stability, reduces aggregation in culture media and on plant surfaces, and facilitates cellular uptake via clathrin-mediated endocytosis. Once internalized, the nanoparticles exploit the plant's membrane trafficking pathways to translocate across tissues, including vascular bundles. Lysozyme contributes additional functionality by enzymatically degrading polysaccharides in the plant cell wall, thereby increasing nanoparticle permeability through the apoplast. This modular platform enables targeted delivery to roots, leaves, pollen, and callus tissues in both monocot and dicot species, offering a non-transgenic route for gene modulation and trait enhancement.

Proof of concept

The efficacy of the LDH nanoparticle delivery system has been validated across multiple plant species, including ***Arabidopsis spp.***, ***Nicotiana spp.***, **tomato, and sorghum**.

- Lysozyme-coated LDH nanoparticles loaded with **GFP mRNA** demonstrated robust translation of the transgene in root tissues, with fluorescence intensities exceeding 200-fold above background levels.
- **Delivery of plasmid DNA encoding GFP** resulted in systemic expression across root, stem, and leaf tissues, including translocation across graft junctions when a mobile tRNA motif was included in the 3' UTR.
- **RNA interference** was successfully induced using siRNA and dsRNA-loaded nanoparticles, with up to 72% reduction in target gene expression in transgenic lines.

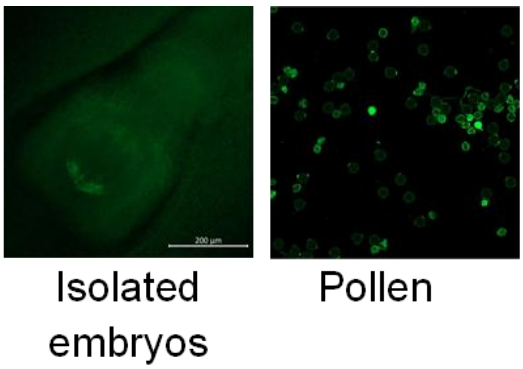

Figure 1: GFP expression visualised in pollen and isolated embryos from plants treated with protein coated LDH + 35s:tRNA:GFP construct.

- Confocal microscopy and flow cytometry confirmed **nanoparticle internalization and transgene expression**, while inhibitor studies (e.g., Wortmannin and Brefeldin-A) validated the role of **endocytosis** and **vesicle trafficking** in nanoparticle uptake.
- The platform also demonstrated compatibility with **hydroponic** and **foliar** delivery and was effective in both intact plants and excised tissues.
- **PCR-amplified transgene fragments** delivered via LDH nanoparticles exhibited higher expression levels than equivalent plasmid constructs, suggesting enhanced transcriptional accessibility.

These results collectively establish the platform as a high-efficiency, non-GM delivery system for functional nucleic acids in plants.

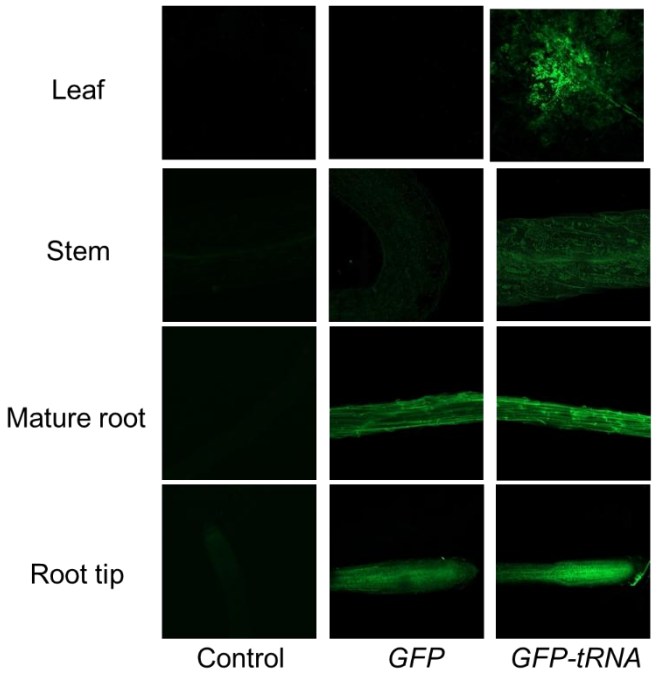

Figure 2: GFP biodistribution in Arabidopsis after delivery of 35s:GFP or 35s:tRNA:GFP constructs into plant roots.

Intellectual property

Patent application PCT/AU2024/050554 entitled "Protein-coated LDH nanoparticles as nanocarriers in plants" claiming a composition comprising nucleic acids adsorbed onto layered double hydroxide (LDH) nanoparticles coated in positively charged protein, used to introduce said nucleic acids to plants and plant tissues.

The system's modularity allows for **customization** of nanoparticle size, protein coating, and nucleic acid payload, making it adaptable to diverse crop species and developmental stages.

Commercialisation opportunities

UniQuest is seeking a commercialisation partner for this protein-coated LDH nanoparticle technology in any of the following applications:

- **Non-GM Trait Delivery Platform**: Enabling transient expression of functional nucleic acids without genomic integration; non-transgenic trait enhancement and gene modulation.
- **RNAi-Based Crop Protection:** Topical or root-based delivery of RNA interference molecules to silence pest/pathogen genes.
- **Gene Editing Reagent Delivery**: Compatible with CRISPR-Cas systems (e.g., sgRNA, Cas9 mRNA), allowing targeted genome editing in intact plant tissues without tissue culture or regeneration.
- **Systemic Transgene Expression**: Delivery of mRNA with mobile tRNA motifs enables vascular translocation and systemic protein expression across graft junctions.
- **High-Throughput Phenotyping & Screening**: Rapid testing of gene function via transient expression, ideal for screening candidate genes in breeding and functional genomics programs.

Research leader

Emeritus Professor Bernard Carroll
School of Chemistry & Molecular Biosciences

Professor Bernard Carroll was appointed to a senior lectureship at the University of Queensland in 1993. His laboratory focuses on using plants as model genetic organisms. He is particularly interested in genes involved in intercellular RNA signalling, and how these affect plant development and plant defence against viruses and transposons. He is also investigating the role of intron splicing in regulation of gene silencing and gene expression, and the role of epigenetics in pollen development.

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