

Nanomaterials Enable Delivery of Genetic Material Without Transgene Integration in Mature Plants

Genetic engineering of plants is at the core of sustainability efforts, natural product synthesis, and agricultural crop engineering. The plant cell wall is a barrier that limits the ease and throughput with which exogenous biomolecules can be delivered to plants. Current delivery methods either suffer from host range limitations, low transformation efficiencies, tissue regenerability, tissue damage, or unavoidable DNA integration into the host genome. Here, we demonstrate efficient diffusion-based biomolecule delivery into tissues and organs of intact plants of several species with a suite of pristine and chemically-functionalized high aspect ratio nanomaterials [1]. Efficient DNA delivery and strong protein expression without transgene integration is accomplished in mature *Nicotiana benthamiana*, *Eruca sativa* (arugula), *Triticum aestivum* (wheat) and *Gossypium hirsutum* (cotton) leaves and arugula protoplasts [2]. Notably, we demonstrate that transgene expression is transient and devoid of transgene integration into the plant host genome, of potential utility for easing regulatory oversight of transformed crops as genetically modified organisms (GMOs) [3]. We demonstrate that our platform can be applied for CRISPR-based genome editing for transient expression of Cas9 and gRNAs. We also demonstrate a second nanoparticle-based strategy in which small interfering RNA (siRNA) is delivered to mature *Nicotiana benthamiana* leaves and effectively silence a gene with 95% efficiency. We find that nanomaterials both facilitate biomolecule transport into plant cells, while also protecting polynucleotides such as RNA from nuclease degradation. DNA origami and nanostructures further enable siRNA delivery to plants at programmable nanostructure loci [4], which we use to elucidate force-independent transport phenomena of nanoparticles across the plant cell wall [5]. Our work provides a tool for species-independent, targeted, and passive delivery of genetic material, without transgene integration, into plant cells for diverse plant biotechnology applications.

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