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

Markita Landry
Chan-Zuckerberg Bio hub Investigator
Department of Chemical and Biomolecular Engineering
University of California, Berkeley

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Abstract:

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. 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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2. Demirer, G.S., Zhang, H., Matos, J., Goh, N., Cunningham, F.J., Sung, Y., Chang, R., Aditham, A.J., Chio, L., Cho, M.J., Staskawicz, B., **Landry, M.P.** High Aspect Ratio Nanomaterials Enable Delivery of Functional Genetic Material Without DNA Integration in Mature Plants. *Nature Nanotechnology* (2019). DOI: 10.1038/s41565-0190382-5NNANO-18081684.
3. **Landry, M.P.‡,** Mitter, N.‡ How Nanocarriers Delivering Cargoes in Plants can Change the GMO Landscape. *Nature Nanotechnology* 2019, 14; pp. 512–514.
4. Zhang, H.*, Demirer, G.S.*, Ye, T., Goh, N.S., Aditham, A.J., Cunningham, F.J., Fan, C., **Landry, M.P.** Low-dimensional DNA Nanostructures Coordinate Gene Silencing in Mature Plants. *PNAS* (2019). DOI: 10.1073/pnas.1818290116.

Biography:

Markita Landry is an Assistant Professor in the Department of Chemical and Biomolecular Engineering at the University of California, Berkeley. She received a B.S. in Chemistry, and a B.A. in Physics from the University of North Carolina at Chapel Hill, a Ph.D. in Chemical Physics and a Certificate in Business Administration from the University of Illinois at Urbana-Champaign, and completed an NSF postdoctoral fellowship in Chemical Engineering at the Massachusetts Institute of Technology.

Her current research centers on the development of synthetic nanoparticle-polymer conjugates for imaging neuromodulation in the brain, and for the delivery of genetic materials into plants for plant biotechnology applications. The Landry lab exploits the highly tunable chemical and physical properties of nanomaterials for the creation of bio-mimetic structures, molecular imaging, and plant genome editing. She is also on the scientific advisory board of Terramera, Inc, and on the scientific advisory board of Chi-Botanic. She is a recent recipient of 18 early career awards, including awards from the Brain and Behavior Research Foundation, the Burroughs Wellcome Fund, The Parkinson's Disease Foundation, the DARPA Young Investigator program, the Beckman Young Investigator program, the Howard Hughes Medical Institute, is a Sloan Research Fellow, an FFAR New Innovator, and is a Chan-Zuckerberg Biohub Investigator.

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