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NSF GRFP Graduate Research Plans Statement

Augmenting Plant Biotechnology by Elucidating Structure-Function Relationships of Nanomaterials in Plants

Keywords: *plant biotechnology, gold nanoparticles, photoporation, structure-function paradigm*

Hypothesis: Developing structure-function relationships of nanomaterials in plants can elucidate rational design principles for plant genetic engineering tools that are superior to current methods.

Background: Plant genetic engineering is poised to strengthen the agricultural, pharmaceutical, and energy industries in the face of climate change and global population growth. Examples include sustainable crops, efficient biofuels, and natural product synthesis. However, traditional biomolecule delivery methods to plants either suffer from low efficiency, narrow host ranges, toxicity, or limited practical applicability due to the transport challenge posed by the plant cell wall [1]. Nanomaterials have highly tunable physicochemical properties that can be harnessed to address these limitations but the scarcity of nanosystems applied to plants has hindered the development of practical relationships between nanomaterial properties and their ability to load and deliver bio-cargo. **Project Motivation:** Gold nanoparticles (AuNPs) are ideal for developing such a structure-function paradigm of nanomaterials in plants owing to the facile control they provide over key properties, their established versatility in biological systems, and their facilitation of bio-cargo loading and protection from cellular metabolism [2]. AuNPs also have the potential for passive delivery that would augment throughput and efficacy, while addressing rupture-based toxicity observed in particle bombardment (gene gun) using gold microparticles [1]. Additionally, the data I obtained (**Figure 1**) demonstrate that AuNPs can deliver bio-cargo such as small interfering RNA (siRNA) and plasmid DNA (pDNA) to intact leaves for gene silencing and expression, respectively. Therefore, I propose to investigate the influence of AuNP size, charge, and surface composition on genetic cargo loading and delivery efficiency, cell wall translocation, and toxicity in plants. I will also integrate an emerging technique known as optical injection (photoporation) that has only been preliminarily but promisingly implemented with AuNPs [3] in mammalian systems and has the potential to augment plant cell wall traversal.

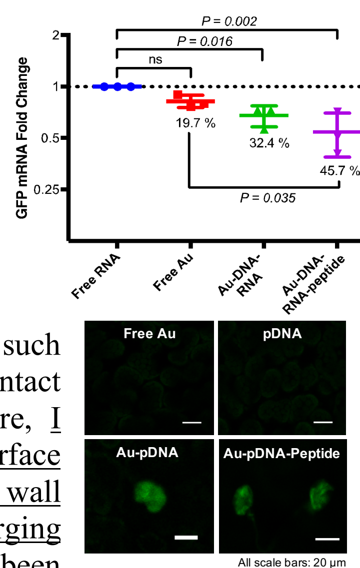

Figure 1. Preliminary silencing and expression results.

Aim 1: Synthesize and characterize a multifactorial AuNP library. Expected outcome: A AuNP landscape that captures an array of physicochemical properties governing nanomaterial-biological system interactions. **Research Plan:** Pristine AuNPs will be modified according to **Table 1**. The size range chosen addresses the disputed range of plant cell wall size exclusion criteria in the literature [2]**.** I will tune surface charge by attaching cationic and anionic polymers at varying amounts, harnessing covalent and noncovalent interactions from my previous work and the literature. The polymers used will have different R groups and backbone geometries to probe AuNP surface composition influence on cell wall translocation and interaction with the intracellular environment. Shape is also key in dictating NP-biological system interactions [4]. I will monitor AuNP colloidal stability with their characteristic absorption spectra, surface charge *via* zeta potential measurements, size *via* dynamic light scattering, and aspect ratio through force and electron microscopy (AFM, TEM).

Table 1. Experimental design space.

Variable	Size (nm)	Surface Charge (mV)	Surface Composition	Shape
Points of Interest	5-50 (5-nm increments)	-40 to 40 (5-mV increments)	Polymer geometry, backbone R groups, cell-penetrating peptides	Sphere, rod, cube

Aim 2: Test AuNP transport, internalization, and toxicity as a function of physicochemical properties and photoporation in intact leaves. Expected outcome: High aspect ratio particles will best traverse the cell wall and highly charged particles will be most toxic. **Research Plan:** For *in vivo* tracking, all AuNPs will be labeled with a fluorophore according to previous methods [5]. Upon needleless syringe infiltration of NPs into leaves, confocal laser scanning microscopy will probe transport and internalization. I will measure differentially expressed genes linked to stress and toxicity *via* RNA sequencing to evaluate plant health. Subcellular NP localization is necessary for certain delivery applications, but the mechanism is not well-understood. As this will depend on the uptake pathway [1], I will use inhibitors of different endocytosis pathways to investigate mechanisms of internalization and subcellular localization as a function of NP properties. All experiments above will also be conducted with experimentally-optimized photoporation conditions (**Figure 2**). This technique has facilitated exogenous cargo transport in mammalians system, where living cells are exposed to a focused, high energy laser pulse that generates a transient hole in the cell membrane, but it has not yet been applied to plant systems.

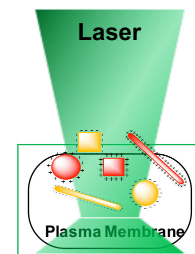

Figure 2. optical injection (photoporation).

Aim 3: Probe bio-cargo loading and delivery efficacy as a function of AuNP properties. Expected outcome: An interplay between toxicity of highly-charged particles and their loading and delivery efficiencies. **Research Plan:** I will load siRNA, pDNA, and ribonucleoproteins such as CRISPR-Cas9 tools onto AuNPs by harnessing covalent and noncovalent methods. Gel electrophoresis and absorption spectroscopy will facilitate cargo loading efficiency optimization and colloidal stability monitoring, respectively. Importantly, if AuNP properties drastically change upon cargo loading, Aim 2 experiments would be repeated with cargo. Prior to administration into plants, I will study the effects of laser conditions optimized in Aim 2 on AuNP-cargo integrity with a Bioanalyzer for nucleic acids and in vitro cutting assays for Cas9 ribonucleoproteins. In plants, initial testing will employ model plants and generic genetic reporters to elucidate NPs with optimal delivery profiles followed by testing more practical constructs in non-model plant species. Gene silencing and expression readouts will be obtained *via* confocal microscopy, quantitative polymerase chain reaction, and Western and Northern blots. To probe the major hypothesis of the proposal, experiments will be performed in parallel with two established methods to evaluate the ability of the developed structure-function model to yield superior plant genetic engineering tools.

Intellectual Merit: The two chief plant genetic engineering methods have not been updated since their advent in the 1980s. With *Agrobacterium* transformation, it can take weeks or months to test a genetic plant variant, and transgenic DNA is integrated into the plant host genome, subjecting it to strict GMO regulations. With gene gun methods, efficiency and toxicity remain as main limitations. This work will harness the level of control we have over nanomaterials as delivery vehicles to make an interdisciplinary impact on high-throughput screening of desired genotype-phenotype combinations, mapping and optimization of plant biosynthetic pathways, and acceleration of plant-mediated natural product synthesis. **Broader Impact**: Simple and effective plant genetic engineering tools will facilitate sustainable and healthy crops, cheaper medicines, and efficient biofuels across the world in the face of climate change and global population growth. This work will involve underrepresented undergraduate and high school students in the frontier of discovery to inspire them to pursue STEM, just as such experiences have contributed to my trajectory. Additionally, I will post preprints on servers such as *bioRxiv* and publish in open-access journals to enable broad and rapid dissemination of this work. **References:** 1) Cunningham, Goh et. al. *TIBTECH.* 36, 882 (2018). 2) Mirkin et. al. *Angew. Chem. Int. Ed.* 49, 3280 (2010). 3) Li et. al. *Nano Lett.* 15, 770 (2015). 4) Champion et. al. *PNAS.* 103, 4930 (2006). 5) Wong et. al. *Nano Lett.* 16, 1161 (2016).