CHARACTERIZATION OF PEPTOID SINGLE-WALLED CARBON NANOTUBE (SWNT) ASSEMBLIES FOR THE DELIVERY OF CAS9 PROTEIN INTO PLANT CELLS

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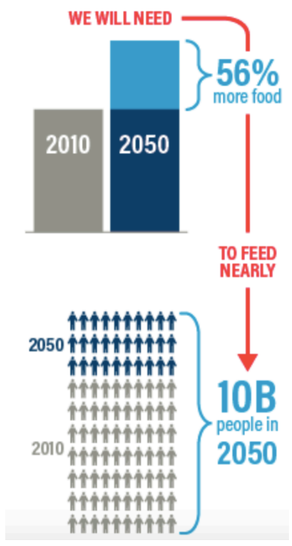
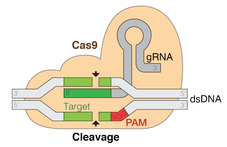
Plant genetic engineering can address several global challenges such as biofuels production, drug discovery, and agricultural development. CRISPR-Cas9 has been developed as a useful tool in target genome editing. In plants, one barrier to CRISPR-Cas9 implementation has been the delivery of Cas9 protein to plant cells, which possess a rigid cell wall. We propose a delivery technology based on peptoid functionalized single-walled carbon nanotubes (SWNTs) conjugated to Cas9 ribonucleoproteins (RNP). Once developed, this system will be tested *in vivo* via infiltration into *Nicotiana benthamiana* leaves. Our research aims to reveal the applications of SWNTs in protein delivery for gene editing, with the hopes that this novel technology can be used in plant science and agriculture.

Introduction



Gene editing CRISPR-Cas9:
RNA guided DNA endonuclease



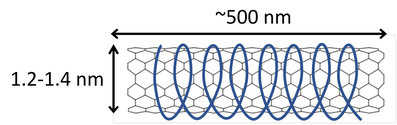
The plant cell wall is rigid and serves as a barrier to exogenous biomolecule delivery

Limitations to Existing Delivery Technology:

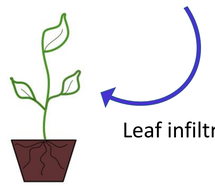
Agrobacterium-mediated delivery
- Can't deliver protein
- Limited range of species

Gene gun-mediated delivery
- Provokes tissue damage
- Limited range of species

OUR TECHNOLOGY:



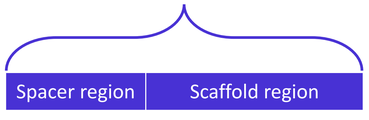
Surface functionalized SWNT



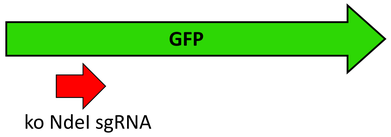
- Single-Walled Carbon Nanotubes (SWNTs) are small, biocompatible, and excellent biomolecule delivery vehicles!
- SWNTs will be delivered to *Nicotiana benthamiana* plant species

METHODOLOGY

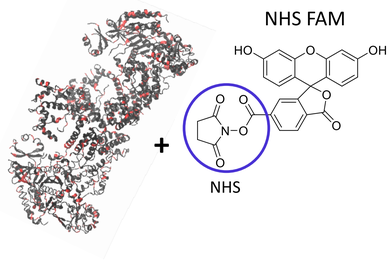
In vitro transcription



Cas9 Activity Assay

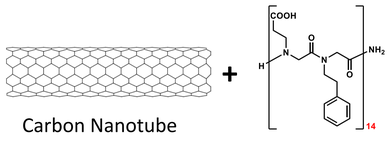


Fluorophore Labeling of Cas9

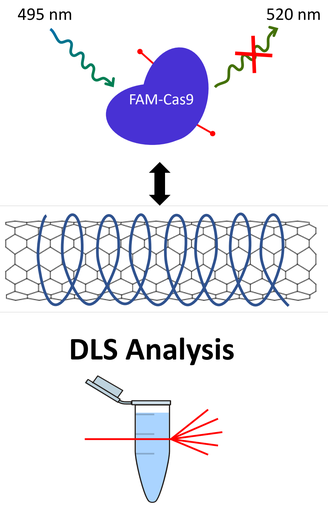


Probe-Tip Sonication

- Using ultrasound and a charged polymer to solubilize pristine SWNTs



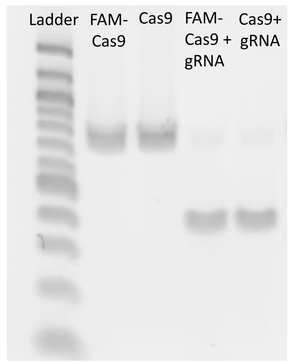
Fluorescence Screen of FAM-Cas9 Peptoid Anchor-SWNT



DLS Analysis

RESULTS

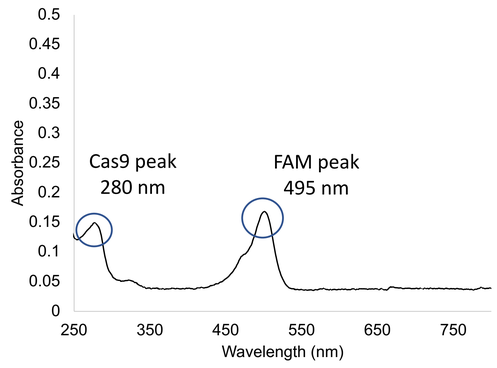
Figure I. Cas9 Activity



	% Cleaved
FAM-Cas9	81.3%
Cas9	80.7%

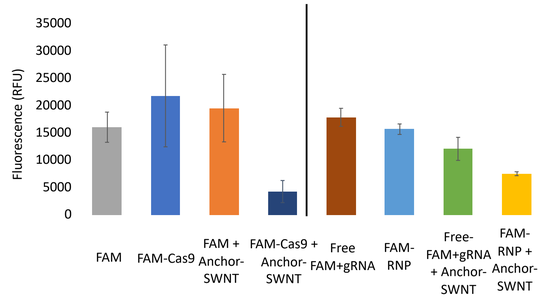
- Labeling did not affect Cas9 activity

Figure II. FAM-Cas9 Absorbance



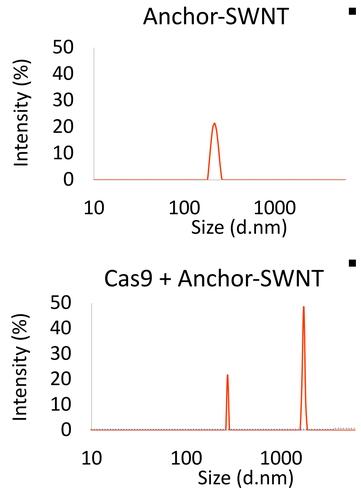
Extent of labeling: ~2.6 dye per protein

Figure III. Average Fluorescence



- A decrease in fluorescence suggests that quenching occurred in the presence of anchor-SWNT

Figure IV. DLS—Size Distribution by Intensity



- The peak at ~300 nm confirms that anchor-SWNTs remain free in solution
- The hydrodynamic radius increased significantly, suggesting aggregates formed when anchor-SWNT was introduced to Cas9 in solution

CONCLUSION

- FAM effectively labels Cas9 without disrupting its function
- Fluorescence assay indicates interactions between Cas9 and Anchor-SWNT
- DLS results indicate that Anchor-SWNT is aggregating in the presence of Cas9 protein
- Cas9 interacts with Anchor-SWNT complex but solution is unstable so peptoid sequence needs to be further optimized

FUTURE WORK

- Synthesize peptoid polymers for binding of Cas9
- Optimize buffer conditions and protein-nanotube ratio
- Leaf infiltration: deliver Cas9 to GFP positive leaves
- Measure GFP gene knockout efficiency

REFERENCES

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